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Moyamoya Disease Explored Through *RNF213*

Genetics, Molecular Pathology, and Clinical Sciences

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Preface

More than 60 years have passed since the first case of moyamoya disease was reported in 1955 by Japanese neurosurgeons. In the 1970s, this unique cerebrovascular occlusive disease was named moyamoya disease, and its distinctive-sounding name, together with the concomitant development of surgical treatments for moyamoya by Japanese surgeons, facilitated its international recognition. Further recognition for moyamoya was earned after the late 1980s by Japanese neurosurgeons through clinical epidemiological studies that suggested the involvement of genetic factors in the development of moyamoya and the demonstration that moyamoya was one of the major causes of stroke in the younger population. All these breakthroughs were made by pioneer neurosurgeon groups.

At the turn of the twenty-first century, I joined a research group on moyamoya disease as an expert of social medicine. The group was supported by the Ministry Health and Labor of Japan and organized by Dr. Nobuo Hashimoto, professor emeritus of Kyoto University and one of the most prominent neurosurgeons in Japan. He expected me as a social medicine researcher to expand the investigations on the genetic factor(s) involved with moyamoya in collaboration with neurosurgeons. Fortunately, in 2010 we were able to identify *RNF213* R4810K (mysterin) as a genetic factor for moyamoya disease. (Please see Chapter 1 for the official report by this group.) However, elucidation of this genetic factor unveiled unexpected complexities: given that the carrier prevalence of *RNF213* R4810K is 0.8–2.0% in the general population in East Asian countries, there is a large gap between the prevalence of moyamoya patients (roughly 1/10000 population) and carriers (1/50 in the general population). This discrepancy required a rational explanation and led us to postulate that there were additional factors, i.e., non-genetic factors, such as environmental factors or infection, for the development of moyamoya disease.

Such a gap also strongly implies that prevention of moyamoya disease can be best achieved by a high-risk strategy focusing on carriers. On the other hand, it raises additional unresolved questions: what is the health risk of R4810K for carriers, what is the function of RNF213, how does R4810K impair RNF213 functions, and how does it lead to moyamoya disease in concert with non-genetic factors?

Addressing such unresolved questions is not only interesting, but also critical for developing nonsurgical therapeutic approaches and disease-prevention strategies. Those unsolved mysteries for *RNF213* need further expansion on the horizon of moyamoya disease with the combined efforts of molecular biologists and biochemists.

The aim of the present book is to provide the most up-to-date information on the role of *RNF213* in moyamoya disease, not only for neurosurgeons, but also for public health researchers and basic biological scientists. Special emphasis, therefore, has been placed on a more comprehensive approach towards the clinical aspects, genetic epidemiology, and biochemical and physiological functions of RNF213. Contributors are well-known frontline clinical, social medicine, and molecular biology researchers of moyamoya disease and *RNF213* in different disciplines beyond neurosurgery.

Current topics in our journal, i.e., *Environmental Health and Preventive Medicine,* published in partnership with the Japanese Society of Hygiene, aim to deliver cutting-edge information as explored by frontline experts from the around the globe in a diverse area of expertise, focusing on prevention of diseases and environmental health related to health risks. The book series is a valuable resource to researchers, clinicians, and administrators who are seeking comprehensive information on environmental health and health promotion. Therefore, I am very proud of this book, not only as a researcher in social medicine, but also as the president of the Japanese Society of Hygiene, because I am certain that our society can contribute to the prevention of moyamoya disease and clearly demonstrate a pivotal role of social medicine in modern health issues. Finally, all the contributors hope that this book stimulates research on vascular diseases through the study of *RNF213* and paves the way to novel innovations for the prevention, treatment, and management of cerebrovascular diseases.

On the behalf of all the contributors and editors.

Kyoto, Japan Akio Koizumi

Contents

Part I
Prologue

Chapter 1
A Prologue to Moyamoya Disease and *RNF213*

Akio Koizumi and Shohab Youssefian

Abstract Almost 20 years after the first case reported in 1955, moyamoya disease (MMD) was still thought to be specific to Asian populations. During the past 60 years, however, MMD has become globally recognized and tremendous therapeutic advances have been made. In 2011, *RNF213* (mysterin) was identified as the major susceptibility gene for MMD. RNF213 is a large protein (591 kDa) and contains both AAA+ ATPase and E3 ligase domains. The p.R4810K variant, a founder mutation common to East Asians, is found at a rate of 70–90% in Japanese and Korean MMD patients and even shows a 1–3% allelic frequency in the general Japanese and Korean populations. As only a minor proportion (approx. 1 out of 200) of carriers develop MMD, environmental factors are thought to be major contributors to the progress of MMD. Recent in vitro molecular studies have revealed that p.R4810K induces endothelial dysfunction, which may ultimately lead to arterial stenosis and development of moyamoya vessels. Furthermore, RNF213 is found to be involved in interferon (IFN), noncanonical *wnt,* and PTP1B/α-ketoglutarate-dependent dioxygenase signal cascades. Such pathways are known to be activated in response to environmental stress signals, such as infection, inflammation, or hypoxia, which may therefore be involved in promoting the development of MMD.

Keywords Moyamoya • *RNF213* • p.R4810K • Inflammation • Hypoxia

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1.1 History of the Discovery of Moyamoya Disease

1.1.1 Dawn of Moyamoya Disease

The first case of moyamoya disease (MMD) was reported by Shimizu and Takeuchi in 1955 at the 14th Annual Meeting of Japan Neurosurgical Society [1], followed by a case with "bilateral hypoplasia" of the internal carotid artery in 1957 [2]. In 1963, Suzuki et al. reported six cases of MMD and established the clinical entity of MMD [3], pointing out that the moyamoya vessels, which displayed a fuzzy appearance similar to a "puff of smoke," were collaterals that compensated for the decreased blood flow resulting from stenosis at the Circle of Willis. In 1968, Kudo suggested that the prevalence of MMD was high in the Japanese population [4].

More complete pathological studies were reported in 1984 by Yamashita et al. [5], who demonstrated that the observed arterial stenosis was a consequence of intimal thickening. However, Ikeda et al. (1991) confirmed that intimal thickening was not limited to the internal carotid artery but occurred in systemic arteries [6].

In 1969, Suzuki and Takaku [7] named the disease as MMD based on two paradigms/concepts: the collateral vessels appeared fuzzy and resembled a "puff of smoke" in angiography and the pathogenesis, prognosis, and epidemiology of MMD were completely obscure at that time and in quite a state of "moyamoya" in Japanese. Currently, this name is well accepted internationally and is formally designated as such in the International Classification of Diseases (ICD) 10.

1.1.2 Emergence of Moyamoya Disease as a Globally Recognized Cerebrovascular Disease

While MMD was globally recognized by the early 1970s, through the development of diagnostic modalities, such as computerized tomography and diagnostic imaging, there was no major progress in MMD therapy. However, Karasawa et al. (1978) developed an epoch-making surgical intervention for revascularization [8]. Through this direct bypass procedure, which included superficial temporal artery to middle cerebral artery anastomosis, cerebral hemodynamics could be normalized and the risk of stroke drastically reduced, thereby contributing to improved prognosis. In 1997, the first clinical guidelines for MMD were published by the Research Committee on Spontaneous Occlusion of Circle of Willis (Moyamoya Disease).

1.2 Road to Genetic Studies Identifying *RNF213* R4810K

Enhanced recognition of MMD among neurosurgeons emerged worldwide as patients, not only in Japan but also in the USA and Europe, were diagnosed with the disease. However, epidemiological studies revealed that the prevalence of MMD per

10^5 population was much higher in East Asian countries, i.e., 10.5 in Japan [9], 9.0 in Korea, and [9] 3.9 in China [10], than in Western countries, for example, 0.086 in the USA [11]. This large difference in prevalence was attributed to a putative common genetic factor in East Asians, and, accordingly, 10–15% of Asian patients were found to have family histories. Such lines of evidence strongly suggested the involvement of genetic factor(s) in the development of MMD in East Asian populations, stimulating genetic studies into MMD. The need for such analysis was timely met by recent developments in genetic analysis techniques. Furthermore, success in the genetic analysis of moyamoya syndrome, in which MMD was associated with various monogenic diseases, encouraged the genetic dissection of MMD [12].

Early studies revealed five potential genetic loci in Japanese patients: 3p24-p26, 6q25, 8q23, and 17q25 [12]. However, the identification of such multiple loci among Japanese patients was unexpected, as a common genetic factor among these patients had been postulated by many researchers. These initial studies, however, suffered from a serious theoretical flaw, known as the "skipping generation phenomenon," which resulted from the low penetrance of MMD. To compensate for this phenomenon, the carrier had to be treated as an "affected" individual, i.e., assuming a "carrier state" that would include cases with various vascular abnormalities, such as stenosis only or unilateral moyamoya. This problem was thereby resolved in 2007 by Mineharu et al. who identified a single locus on 17q25.3. Further details of these studies are available in our recent review [12].

Three years after these rigorous linkage studies, the RING finger protein 213 gene (*RNF213*, i.e., mysterin) was identified as the major susceptibility gene for MMD. The *RNF213* variant p.R4810K (c.14429G>A, rs112735431, ss179362673, or simply R4810K hereafter) was first reported by the Kyoto group, with a high level of association (odds ratio 63.9, 95%, confidence interval 33.9–120.4) [13], and later confirmed by an association study [14] and familial linkage analysis with exome sequencing [15]. The R4859K [14] and R4810K [15] variants identified in these studies were found to correspond to rs112735431. Genetic epidemiology demonstrated that R4810K is a common founder mutation among East Asian patients as initially postulated: R4810K carrier prevalence is 90.1% in Japanese patients, 78.9% in Korean patients, and 23.1% in Chinese patients [15]. Interestingly, R4810K is also found in the general population at rates of 2.5% in Japanese, 2.7% in Korean, and 0.9% in Chinese [15], as also confirmed by later studies (see review [12]). Although the R4810K mutation has not yet been identified in Caucasian patients (see review [12] and [15]), various polymorphisms in both East Asian and Western populations (Fig. 1.1a) have been reported [12] as of 2016.

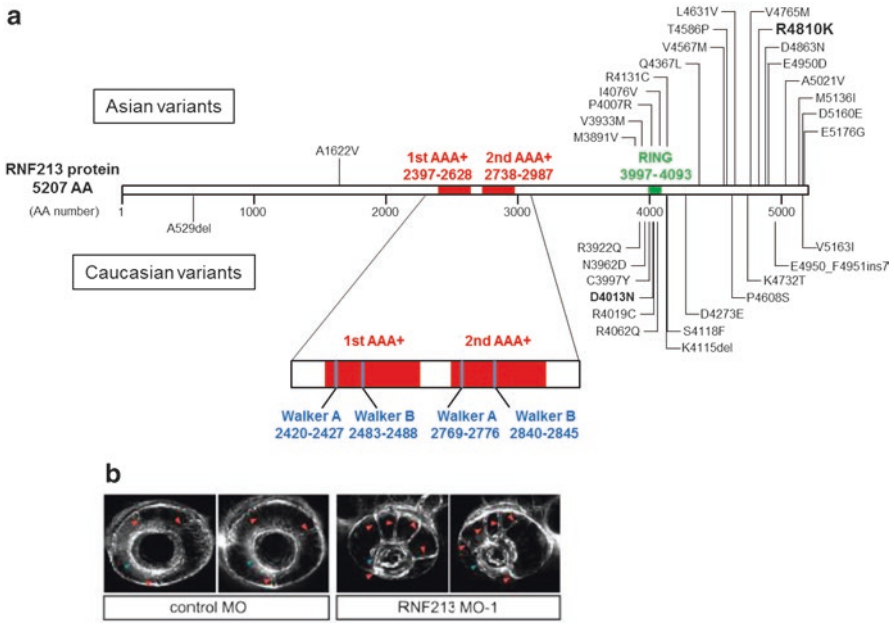

Fig. 1.1 *RNF213* structure and mutations, and negative effects of RNF213 suppression on ocular arteries. (**a**) Structure and mutations of *RNF213* found in patients with moyamoya cited from [12]. (**b**) Extraocular arteries in zebrafish embryos 72 h after administration of morpholino oligomer to inhibit RNF213 (Cited from [15])

1.3 *RNF213* and Moyamoya Disease

The study by Liu et al. [15] was the first to clone the full-length RNF213 cDNA, to characterize RNF213 biochemically and demonstrate that it has both E3 ligase and ATPase activities (Fig. 1.1a). They also showed that inhibition of RNF213 expression induced an abnormal development of ocular arteries in zebrafish (Fig. 1.1b).

1.3.1 Molecular Characteristics of RNF213

RNF213 is composed of 5207 amino acids with a molecular mass of 591 kDa. Morito et al. [16] demonstrated that RNF213 has two AAA+ modules that are involved in the formation of a hexameric ring: oligomerization is initiated by ATP binding by the Walker A motif of the first AAA+ module, whereas ATP hydrolysis by the Walker B motif relaxes the oligomeric complex. The physical movement of the hexameric ring, through rhythmical ATP binding and hydrolysis, is thought to modify substrate(s) interacting with the hexamer. RNF213 has an additional RING domain, encoding an E3 ligase, which is involved in the ubiquitination of substrates targeted for either proteosomal degradation or signal transduction.

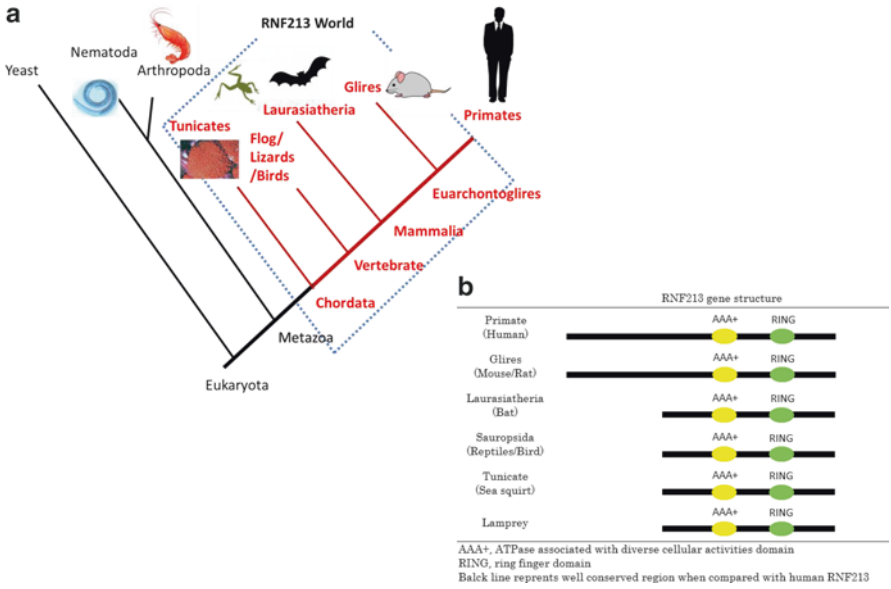

Fig. 1.2 Evolutional development of *RNF213*. (**a**) Phylogenic tree [17]. (**b**) Module conservation in evolutionary trees [17]

RNF213 orthologues are found in biota higher than the animal phylum, Chordata (Fig. 1.2a) [17]. The *RNF213* evolutionary tree (Fig. 1.2a) provides clues to the evolutionary pathway of *RNF213*, in which an N-terminal region with the AAA+ domain combined with a C-terminal region containing the E3 ligase domain. Phylogenic analysis indicates that vertebrate *RNF213* possesses both AAA+ and E3 ligase domains, including lampreys, representatives of an ancient vertebrate lineage, and the *Ciona* sea squirt, a tunicate (Fig. 1.2b). Comparisons among biota may imply that C-terminal portion of *RNF213* may be more functionally essential than N-terminal one.

1.3.2 Molecular Pathology of RNF213

RNF213 mutations in MMD patients reported to date are all missense mutations or in-frame insertions or deletions and cluster around the RING finger domain and C-terminal portion of *RNF213* rather than in its AAA+ domain and N-terminal region (Fig. 1.1a).

Hitomi et al. established iPS cells (iPSCs) from patients carrying R4810K, successfully differentiated them into vascular endothelial cells (ECs), and proved that these Ecs have impaired angiogenic functions, based on tube formation and migration assays, and also display mitotic abnormalities [18].

Kobayashi et al. [19] and Ohkubo et al. [20] independently demonstrated that specific cytokines upregulated *RNF213* expression. Interferons (IFNs) are one such group of cytokines, which are also well known to inhibit angiogenesis, and Kobayashi et al. [19] provided clear evidence that this inhibition was mediated through RNF213.

These data collectively suggest that RNF213 upregulation in response to IFNs and also R4810K expression induce EC dysfunction and thereby have an inhibitory effect on angiogenesis. Such EC dysfunction, with subsequent proliferation of smooth muscle cells, may explain the vascular constriction at the end of the internal carotid artery in patients reported by Kaku et al. [21]. However, no study so far has successfully demonstrated a pathological consequence of R4810K on stenotic lesion formation around the circle of Willis in in vivo models.

1.3.3 Involvement of RNF213 *in Diverse Biological Processes*

Recent studies have revealed that *RNF213* is associated with various signal transduction pathways, such as INF and STAT [19, 20], noncanonical *wnt* signaling [22], and a *PTP1B* /α-ketoglutarate-dependent dioxygenase axis [23].

This latter pathway, which has emerged as a novel signal transduction system that may link vascular and metabolic phenotypes, implies that RNF213 may regulate non-mitochondrial oxygen consumption in *Her2*$^+$ breast cancer cells [23]. The transcription factor HIF-1 is well known as a major oxygen sensor that decreases oxygen consumption in mitochondria under hypoxia (oxygen concentrations of 1–10%). Under more severe hypoxia (oxygen concentrations as low as 0.1%), however, non-mitochondrial oxygen consumption turns out to be the primary oxygen sink and one that is mainly regulated by a protein tyrosine phosphatase, PTP1B, through RNF213 and α-ketoglutarate-dependent dioxygenases [23]. This process minimizes oxygen consumption in the non-mitochondrial fraction and thereby provides cancer cells with an adaptive strategy through which they can survive severe hypoxic stress. When *PTP1B* is ablated, *RNF213* is constitutively activated, and the dysregulated non-mitochondrial oxygen consumption reduces oxygen availability and leads to hypoxia-induced death of the cancer cells. Such novel functions of *RNF213* in *Her2*$^+$ cells under severe hypoxia may be indicative of the role that RNF213 is thought to play in angiogenesis under hypoxia as reported by Kobayashi et al. [19].

1.3.4 Putative Signal Transduction (Fig. 1.3)

As discussed above, upregulation of RNF213 by IFN or overexpression of R4810K, but not overexpression of wild-type RNF213, in ECs has been shown to impair cellular angiogenic potential [19]. These findings suggest that IFNs, in addition to

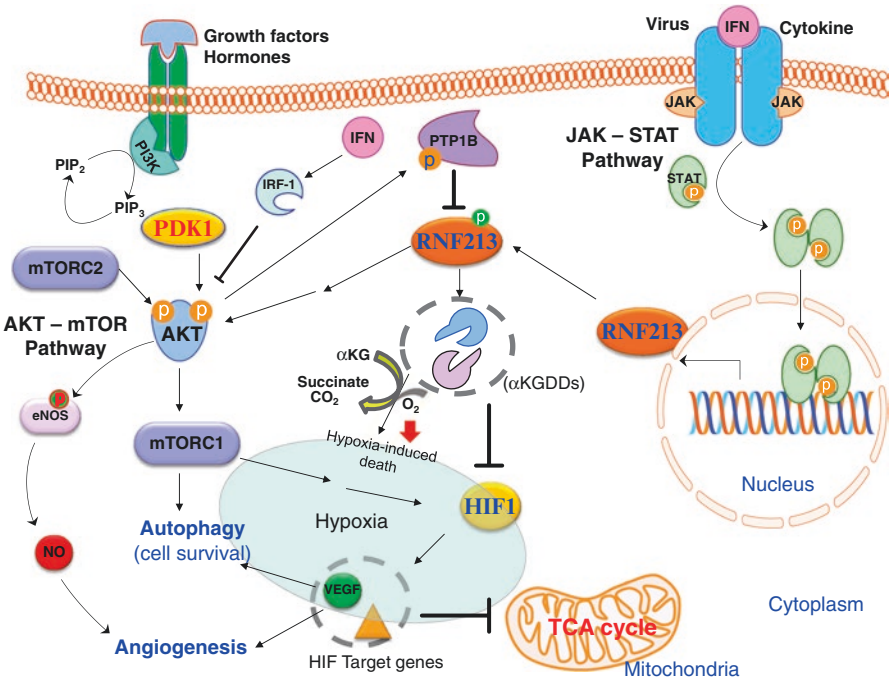

Fig. 1.3 RNF213 involved in multiple putative signal cascades. Refer to the text for details

upregulating RNF213 expression, also modify other signaling pathways that impact EC functions. One possibility is the AKT-mTOR signal pathway, which is known to play key roles in cellular functions as diverse as angiogenesis, autophagy, glucose metabolism, cell migration, and invasion. Indeed, AKT phosphorylation, and also angiogenesis, is found to be inhibited by IFN-induced IRF-1 [24] as well as by *RNF213* knockdown in ECs [20] and to be essential for hypoxia-induced activation of HIF-1 and subsequent VEGF-induced stimulation of angiogenesis or autophagy [25]. Interestingly, AKT is also known to phosphorylate PTP1B [26]. Furthermore, the AKT pathway has been implicated eNOS production and the subsequent regulation of angiogenesis through NO signaling in ECs [27]. Finally, mTOR is also known to be suppressed by severe hypoxia and to play a critical role in autophagy, i.e., cell survival under stress conditions.

While speculative, we currently consider that *RNF213* R4810K may downregulate the AKT-mTOR pathway and, through changes in autophagy, particularly under hypoxic conditions, impair EC functions. Similarly, WT RNF213, when stimulated by IFN, may also deactivate the AKT-mTOR pathway and lead to a comparable dysfunction in ECs, thereby leading to smooth muscle cell proliferation, arterial stenosis, and the resulting MMD phenotype.

1.4 Future Perspectives

It is surprising that a case with a rare cerebrovascular abnormality found in 1955 has opened the door to the now globally recognized moyamoya disease. Although the identification of *RNF213* provides us with the unique opportunity of dissecting MMD from a molecular aspect, yet new questions have arisen that must be addressed through strategic approaches. For example, do environmental factors impact the low penetrance of *RNF213*, which signal pathways modulate RNF213 action, how does RNF213 lead to vascular stenosis, and what are the possible health risks for the 15 million East Asians that are R4810K carriers?

The first research approach is to identify the environmental factors that may affect RNF213 expression. So far, neither ablation of *RNF213* nor R4810K expression in knock-in mice or overexpression in transgenic mice shows any discernible vascular phenotypes under normal conditions, although the transgenics do display lowered angiogenesis under hypoxia [19]. Therefore, R4810K expression alone may not be sufficient to induce the modified vascular phenotypes and may require specific environmental inducers, such as infection, inflammation, or hypoxia. These environmental signals need to be identified.

The second research approach is to elucidate the signal transduction cascades associated with RNF213. Although RNF213 is known to be involved in numerous biological processes (Fig. 1.3), our understanding of the RNF213 involvement in these pathways is still quite rudimentary.

The third approach is to elucidate the molecular functions of RNF213, through its AAA+ ATPase and E3 ligase activities, and thereby identify its direct substrates and cofactors and the various pathways in which it plays a role.

The fourth approach is an epidemiological one. It is quite important to identify the health risks for R4810K carriers on a population basis. Although there are several reports that suggest health risks of carriers (e.g., hypertension, renovascular hypertension, coronary heart disease, or cerebrovascular stenosis) [12], no large-scale epidemiological study has ever been reported.

Such strategic approaches will provide us with a more comprehensive understanding of *RNF213*, its role in vascular stenosis, and how the cerebral vascular system adapts to environmental stress. Such information is essential for establishing an effective preventative strategy for cerebrovascular diseases.

References

1. Shimizu K, Takeuchi K. Bilateral hypoplasia of internal carotid arteries. (in Japanese). The 14th Annual meeting of the Japan Neurosurgical Society; 1955.
2. Takeuchi K, Shimizu K. Hypoplasia of bilateral internal carotid arteries. (in Japanese). Brain Nerve. 1957;9:37–43.
3. Suzuki J, Kowada M, Kyoku H, Takaku A. Clinical investigation on cases presenting unique angiographic images with collateral vessels by angiography (in Japanese). The 22nd Annual meeting of the Japan Neurosurgical Society; 1963.

4. Kudo T. Spontaneous occlusion of the circle of Willis. A disease apparently confined to Japanese. Neurology. 1968;18(5):485–96.
5. Yamashita M, Oka K, Tanaka K. Cervico-cephalic arterial thrombi and thromboemboli in moyamoya disease–possible correlation with progressive intimal thickening in the intracranial major arteries. Stroke. 1984;15(2):264–70.
6. Ikeda E. Systemic vascular changes in spontaneous occlusion of the circle of Willis. Stroke. 1991;22(11):1358–62.
7. Suzuki J, Takaku A. Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20(3):288–99.
8. Karasawa J, Kikuchi H, Furuse S, Kawamura J, Sakaki T. Treatment of moyamoya disease with STA-MCA anastomosis. J Neurosurg. 1978;49(5):679–88. doi:10.3171/jns.1978.49.5.0679.
9. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7(11):1056–66.
10. Miao W, Zhao PL, Zhang YS, Liu HY, Chang Y, Ma J, et al. Epidemiological and clinical features of Moyamoya disease in Nanjing, China. Clin Neurol Neurosurg. 2010;112(3):199–203. doi:10.1016/j.clineuro.2009.11.009.
11. Uchino K, Johnston SC, Becker KJ, Tirschwell DL. Moyamoya disease in Washington state and California. Neurology. 2005;65(6):956–8. doi:10.1212/01.wnl.0000176066.33797.82.
12. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of moyamoya disease and associated health risks explored through RNF213. Environ Health Prev Med. 2015;21(2):55–70. doi:10.1007/s12199-015-0498-7.
13. Koizumi A. Genetic analysis of familial moyamoya. Annual report of the research committee on spontaneous occlusion of the circle of willis (Moyamoya Disease by Science Research Grants of Ministry of Health, Labor and Welfare, Japan (Chaired by N. Hashimoto); 2010.
14. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40. doi:10.1038/jhg.2010.132.
15. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542. PONE-D-10-04031 [pii]
16. Morito D, Nishikawa K, Hoseki J, Kitamura A, Kotani Y, Kiso K, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. Sci Report. 2014;4:4442. doi:10.1038/srep04442.
17. eEnsemblAsiaGenetree. http://asia.ensembl.org/Homo_sapiens/Gene/Compara_Tree?db=core;g=ENSG00000173821;r=17:80260866-80398786. Accessed 26 July 2016.
18. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, 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(1):13–9. doi:10.1016/j.bbrc.2013.07.004. S0006-291X(13)01144-3 [pii]
19. Kobayashi H, Matsuda Y, Hitomi T, Okuda H, Shioi H, Matsuda T, et al. Biochemical and functional characterization of RNF213 (Mysterin) R4810K, a susceptibility mutation of moyamoya disease, in Angiogenesis In Vitro and In Vivo. J Am Heart Assoc. 2015;4(7) doi:10.1161/JAHA.115.002146.
20. Ohkubo K, Sakai Y, Inoue H, Akamine S, Ishizaki Y, Matsushita Y, et al. Moyamoya disease susceptibility gene RNF213 links inflammatory and angiogenic signals in endothelial cells. Sci Report. 2015;5:13191. doi:10.1038/srep13191.
21. Kaku Y, Morioka M, Ohmori Y, Kawano T, Kai Y, Fukuoka H, et al. Outer-diameter narrowing of the internal carotid and middle cerebral arteries in moyamoya disease detected on 3D constructive interference in steady-state MR image: is arterial constrictive remodeling a major pathogenesis? Acta Neurochir. 2012;154(12):2151–7. doi:10.1007/s00701-012-1472-4.

22. Scholz B, Korn C, Wojtarowicz J, Mogler C, Augustin I, Boutros M, et al. Endothelial RSPO3 controls vascular stability and pruning through non-canonical WNT/Ca(2+)/NFAT signaling. Dev Cell. 2016;36(1):79–93. doi:10.1016/j.devcel.2015.12.015.
23. Banh RS, Iorio C, Marcotte R, Xu Y, Cojocari D, Rahman AA, et al. PTP1B controls non-mitochondrial oxygen consumption by regulating RNF213 to promote tumour survival during hypoxia. Nat Cell Biol. 2016;18(7):803–13. doi:10.1038/ncb3376.
24. Lee JH, Chun T, Park SY, Rho SB. Interferon regulatory factor-1 (IRF-1) regulates VEGF-induced angiogenesis in HUVECs. Biochim Biophys Acta. 2008;1783(9):1654–62. doi:10.1016/j.bbamcr.2008.04.006.
25. Domigan CK, Warren CM, Antanesian V, Happel K, Ziyad S, Lee S, et al. Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. J Cell Sci. 2015;128(12):2236–48. doi:10.1242/jcs.163774.
26. Ravichandran LV, Chen H, Li Y, Quon MJ. Phosphorylation of PTP1B at Ser(50) by Akt impairs its ability to dephosphorylate the insulin receptor. Mol Endocrinol. 2001;15(10):1768–80. doi:10.1210/mend.15.10.0711.
27. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature. 1999;399(6736):601–5. doi:10.1038/21224.

Chapter 2
Concept of Moyamoya Disease

Haruto Uchino, Ken Kazumata, and Kiyohiro Houkin

Abstract Moyamoya disease is characterized by progressive stenosis or occlusion of the major cerebral arteries, which often results in cerebral strokes. The concept of moyamoya disease was first proposed in Japan during the late 1960s. Recently, global recognition of this disease has increased. From a global perspective, this disease entity may be more appropriately classified as a syndrome that includes a broad spectrum of diseases of differing etiology sharing similar morphologies of the involved cerebral arteries. Indeed, current diagnosis depends on the morphological features of these cerebral arteries. However, it is often difficult to predict prognosis based upon cerebral artery morphology alone, and biomarkers are required in order to discern the various clinical phenotypes. The susceptibility gene *RNF213* has been identified to have strong association with moyamoya diagnosis in East Asian patients and has proven useful as a clinical biomarker. Nevertheless, the trigger of the disease has yet to be clarified, and susceptibility genes have not been detected in non-Asian patients. Multiple common genetic variants and some secondary factors, including epigenetic factors, contribute to the development of the diverse clinical phenotypes of the disease. Therefore, deep phenotyping by proper stratification of heterogeneous populations using clinical, radiological, and biomarker patterns is essential in order to analyze individual etiological factors.

Keywords Moyamoya • Deep phenotyping • Etiology • Epigenome • Syndrome

2.1 Concept and Pathophysiology

Moyamoya disease (MMD) is characterized by progressive stenosis or occlusion of the terminal branches of the internal carotid arteries (ICAs). The steno-occlusive changes are observed in the proximal portion of the middle and anterior cerebral arteries (MCAs and ACAs). Furthermore, the posterior cerebral arteries (PCAs) are

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sometimes involved, often resulting in poor prognosis. The disease is characterized by the development of an abnormal fine vascular network ("moyamoya vessels") at the base of the brain, which is regarded as a compensatory mechanism that occurs in response to cerebral ischemia, resulting in the dilation of perforating arteries as well as the abnormal formation of collateral networks.

The major clinical presentations of MMD involve cerebral ischemic symptoms due to steno-occlusive lesions of the cerebral arteries and intracranial bleeding due to rupture of the fragile vessels in the compensatory network. Most pediatric patients present with ischemic symptoms such as transient ischemic attack or cerebral infarction, while adult patients exhibit increased rates of intracranial bleeding. Furthermore, chronic cerebral ischemia may result in cognitive dysfunction in both pediatric and adult patients even without history of stroke [1, 2]. Recent developments in magnetic resonance imaging and angiography (MRI and MRA) have afforded clinicians increased opportunities to diagnose asymptomatic MMD [3]. Even asymptomatic patients are known to have a potential risk of cerebral strokes, and evidence suggests that pathology in such patients may not be much different from that in symptomatic patients [4]. Surgical revascularization procedures such as direct and indirect bypasses have been effective in improving cerebral hemodynamic compromises [5]. In particular, indirect bypasses using vascularized donor tissues such as temporal muscles and dura mater tissue are considered specific methods for treating MMD pathology. Revascularizations via indirect bypass are more successful in pediatric patients compared with adults, although the degree of revascularization varies in each patient even when the same surgical methods are utilized [6]. Furthermore, some patients require additional surgeries when conventional surgeries are refractory. The molecular mechanism underlying this difference in the responsiveness to surgery, however, remains to be elucidated.

In 1957, Takeuchi et al. first reported the disease as involving bilateral occlusion of terminal ICAs [7]. In 1969, Suzuki et al. named this entity moyamoya disease and established the concept of the disease internationally [8]. (*Moyamoya* means "puff of smoke" in Japanese, and the name was chosen to describe the appearance of the abnormal vascular network.) Since then, for more than half a century, epidemiological/etiological studies in the field have progressed, along with the development of effective surgical revascularization procedures [3]. Furthermore, international recognition and diagnoses of the disease have continued to increase. According to a PubMed search, 162 papers were published in the first decade following recognition of the disease (1969–1980); however, in the last decade (2005–2015), 1526 papers have been published.

Guidelines for the diagnosis of MMD have been published by the Research Committee of MMD in Japan [5]. However, international diagnostic criteria have not been established. Diagnosis relies on the aforementioned morphological features of cerebral arteries, yet MMD may be more appropriately regarded as a syndrome encompassing a broad spectrum of distinct diseases of varying etiology that share common morphological features of the involved arteries. Discrimination of

cerebral atherosclerosis is sometimes difficult using radiological findings alone, though a recent study of *RNF213* indicates that a particular subset of patients share a common genetic predisposition for MMD [9]. Current diagnostic standards, which rely solely on morphological traits, render preclinical diagnosis for disease prevention and ultra-early diagnosis of initial lesions difficult. Therefore, in terms of clinical significance, research should focus on developing objective diagnostic criteria involving novel biomarkers and/or diagnostic algorithms.

The angiographic features of MMD exhibit dynamic changes during the course of the disease: Initially, steno-occlusive changes in the terminal ICAs are observed, though the lesions may gradually progress depending upon the extent of the compensatory collateral vessels from the external carotid arteries [8, 10]. These initially developed moyamoya vessels at the base of the brain then disappear, as in a "puff of smoke." In some cases, these steno-occlusive changes are observed unilaterally. Classical Suzuki's staging well represents this temporal profile of compensatory changes, not the clinical severity of the disease, which is sometimes misunderstood. Prediction of the severity or prognosis only by angiographic features remains limited. Indeed, a subset of patients exhibit malignant clinical courses, though most newly diagnosed patients are classified in Suzuki's stage 3 or 4. A new grading system that takes hemodynamic insufficiency into account has been proposed for assessing prognosis in patients with MMD [11]. However, it is often difficult to distinguish patients exhibiting progressive clinical courses from others.

Furthermore, the aforementioned angiographic changes are associated with several other diseases or acquired conditions as well [5, 12]. In such cases, these conditions are referred to as moyamoya syndrome (MMS) or quasi-moyamoya disease. Common MMS-associated diseases include thyroid disease, neurofibromatosis type 1, Down syndrome, cranial irradiation, and sickle cell anemia [13]. This categorization scheme is a historical concept based on the idea that typical MMD is idiopathic. Although the morphological features of cerebral arteries and clinical courses may vary depending on the associated diseases, surgical revascularization is effective in improving cerebral hemodynamic compromises even in MMS [5, 12]. Especially in non-Asian populations, several gene mutations are also known to be associated with MMS (e.g., Noonan syndrome, Costello syndrome, Alagille syndrome, *GUCY1A3* mutations, *SAMHD1* mutations, Majewski syndrome, Seckel syndrome, deletion of *BRCC3/MTCP1*, and *ACTA2* mutations) [14]. The strong association between MMD and *RNF213* mutation observed in East Asian people may therefore be regarded as a kind of MMS. Importantly, some groups of patients with MMD exhibit stenotic lesions of extracranial arteries such as the renal, coronary, pulmonary, pancreatic, or iliac arteries [15]. This suggests that MMD may involve systemic factors that affect the extracranial arteries. The aforementioned findings indicate that the concept or definition of MMD and MMS may change as research in the field continues to progress.

2.2 Epidemiology

As mentioned previously, global recognition of MMD has increased, though research suggests that the prevalence and incidence are predominantly high in East Asian countries such as Japan and Korea [16–18]. In Japan, the female-to-male ratio is approximately 2:1, and the distribution of onset age exhibits a bimodal pattern, with a first peak between 5 and 10 years of age and a second peak at about 40 years of age. Although the precise reason remains unknown, the female predominance and bimodal pattern of onset age suggest the involvement of some hormonal influences. Familial occurrence is noted in about 10–15% of patients, and these familial cases exhibit a higher female ratio and lower onset age than sporadic cases [5].

Although the data for non-Asian populations are limited, these epidemiological features also vary according to race. MMD in Caucasian patients is characterized by higher onset age, lower rate of hemorrhagic stroke in adults, and lower familial occurrence compared with MMD in East Asian patients [19–21]. However, reports indicate that the incidence rate of MMD for people with Asian ethnic origins in the USA is similar to that in Japan [17]. In light of this finding, the regional difference in epidemiological features may be mainly due to genetic factors, though it is likely that unknown environmental factors are also involved. Future collaborative research regarding the epidemiological aspects of MMD around the world may elucidate those factors related to the development and different clinical expressions of MMD.

2.3 Pathological Nature

In MMD, typical histopathological findings in the terminal portion of ICAs include fibrocellular thickening of the intima resulting in stenosis of the vascular lumen, an irregular formation of the internal elastic lamina, and attenuation of the media [22]. These findings are also observed in peripheral arterial walls, and the fragility of the involved structures may result in intracranial bleeding [23]. The thickened intimal layer is predominantly composed of smooth muscle cells (SMCs) and characterized by the absence of inflammatory cells or atherosclerotic changes [24]. Furthermore, reductions in the outer diameter of involved vessels are noteworthy in terms of diagnostics and etiology [3, 25]. The changes in hemodynamics or shear stress due to this decreased vessel diameter may result in intimal thickening by inducing cell accumulation. An abnormal thrombogenesis in the internal wall of moyamoya arteries has also been reported [26], though this may be the result of chronic inflammation rather than the cause of MMD. Nevertheless, several prothrombotic disorders such as sickle cell disease, antiphospholipid syndrome, and protein-S deficiency are known to be associated with MMS [5, 12, 14].

Though the precise cellular abnormalities leading to the development of MMD remain unclear, quantitative and qualitative abnormalities have been observed in endothelial cells (ECs) [27, 28], SMCs [29, 30], and vascular progenitor cells

(VPCs) [12, 14, 31]. The exact origin of the accumulating SMCs in the intimal thickening also remains to be elucidated. These cells may originate from the underlying media, but not necessarily from SMCs. Alternatively, VPCs such as endothelial progenitor cells (EPCs) or smooth muscle progenitor cells (SMPCs) may cause abnormal intimal thickening [32]. EPCs, first identified in 1997, are implicated in vasculogenesis and intimal repair in some pathological conditions [33]. Functional and qualitative abnormalities in EPCs have also been reported in MMD [12]. Recent research has further revealed that SMPCs, first identified in 2002, exhibit differential gene expression in MMD [34]. Furthermore, direct evidence suggests that such progenitor cells accumulate in the intima of terminal ICAs [35]. The tunica adventitia has been identified as a progenitor cell-rich compartment that regulates vascular wall progenitor cells [32], suggesting that the adventitia is involved in some vascular disease conditions and vascular wall pathogenesis. The decrease of vascular diameter in MMD may therefore be linked to abnormalities in the adventitia, and different types of cells/interactions may be involved in the variation in pathogenesis among individual patients.

A number of studies have focused on angiogenesis in MMD, reporting increases in basic fibroblast growth factor (b-FGF) and hepatocyte growth factor (HGF) in cerebrospinal fluid, as well as increases in serum levels of transforming growth factor (TGF)-beta [36–38]. These cytokines may be related to certain elements of MMD pathogenesis, such as endothelial proliferation and the development of abnormal vessels. However, studies to date have not allowed researchers to conclude whether these abnormalities are purely a response to ischemia or a cause of the disease. Recently, however, some research has indicated that differential expression and disruptions in the balance of metalloproteinases (MMPs) and their tissue inhibitors may lead to instability of vascular structures [39, 40].

However, studies utilizing pathological approaches have had difficulty in collecting specimens of vessel stenosis. Though molecular research using induced pluripotent stem cells (iPSCs) and animal models has progressed significantly, stenosis of cerebral vessels has yet to be reproduced.

2.4 Genome, Epigenome, and Other Factors in MMD

The contributions of genetic factors have been assumed since the initial recognition of the disease due to regional differences in occurrence and familial diagnoses. Since the 1990s, many association analyses and linkage analyses have been conducted [12, 14, 41], supporting this initial assumption. In 2011, the susceptibility gene *RNF213* was identified in East Asian patients [42, 43], which greatly advanced the work of the etiologic studies described in other chapters.

On the other hand, *RNF213* alone does not explain several issues. First, RNF213 p. R4810K, which has been strongly associated with MMD in Japanese patients, is absent in Caucasian patients. Genome-wide association studies (GWAS) have failed to identify any significantly associated genes in Caucasians [44]. Therefore, it is

likely that multiple common genetic variants and environmental factors contribute to aspects of the disease. Second, *RNF213* is a susceptibility gene, not a disease-causing gene, for MMD that exhibits low penetration, while specific triggers of the disease remain to be elucidated. Most of the Japanese or Korean patients are heterozygous for RNF213 p. R4810K, yet this subgroup is composed of various phenotypes (e.g., pediatric vs. adult patients, ischemic vs. hemorrhagic, bilateral vs. unilateral, or progressive vs. stable), suggesting that many unknown factors other than *RNF213* contribute to the development and modification of the disease.

Double-hit theory has been used to explain the mechanism of MMD [12, 45]. This theory postulates that genetically susceptible individuals develop MMD when they experience secondary factors. Indeed, several types of infection and certain autoimmune diseases have been reported to be associated with MMD [5, 12]. The latest *RNF213* research also indicates that some inflammation (infection or autoimmune related) is necessary to cause MMD, in addition to *RNF213* mutation [30]. Shear stress may also play an important role, as the stenosis occurs mainly in terminal ICAs, and the velocity of cerebral blood flow increases in several anemias (e.g., sickle cell, aplastic, and Fanconi anemia) and hyperthyroidism [46, 47]. Such research indicates the potential contribution of shear stress in addition to genetic or autoimmune factors in the aforementioned conditions.

Epigenetic factors, such as histone modification, DNA methylation, and noncoding RNAs, are also important for explaining variations in clinical expression in a number of conditions. Recent publications indicate that epigenetic studies of MMD have been initiated [48, 49], which may allow for the development of potential diagnostic and therapeutic targets in the future.

Given the aforementioned research, various factors account for the wide range of phenotypes observed, though the double-hit theory is insufficient in explaining this variation. The “pinball” metaphor may more appropriately describe the complicated aspects of the disease (Fig. 2.1). This model is derived from Waddington’s epigenetic landscape [50], who originally proposed the model to represent how individual cells differentiate into many different cell types during development. In the metaphor, a ball (representing a cell) rolls down a slope to different outcomes or cell fates depending upon the epigenetic phenomena encountered, like a pinball whose fate is determined based upon the bumpers struck during the game.

In order to advance pathogenesis research, it is therefore necessary to properly stratify these heterogeneous patient populations. Deep phenotyping combining clinical, radiological, and biomarker patterns may allow for deeper analysis of drivers or effectors of the disease. As most cases of MMSs occur within stratified subgroups and the associated diseases have well-recognized etiology or pathophysiology, it is possible to explore pathogenic mechanisms in individual MMS. Furthermore, molecular research is expected to ultimately open up a new pathway toward preclinical diagnosis, evaluation of disease activity, prediction of prognosis, and mechanism-specific treatment in the future.

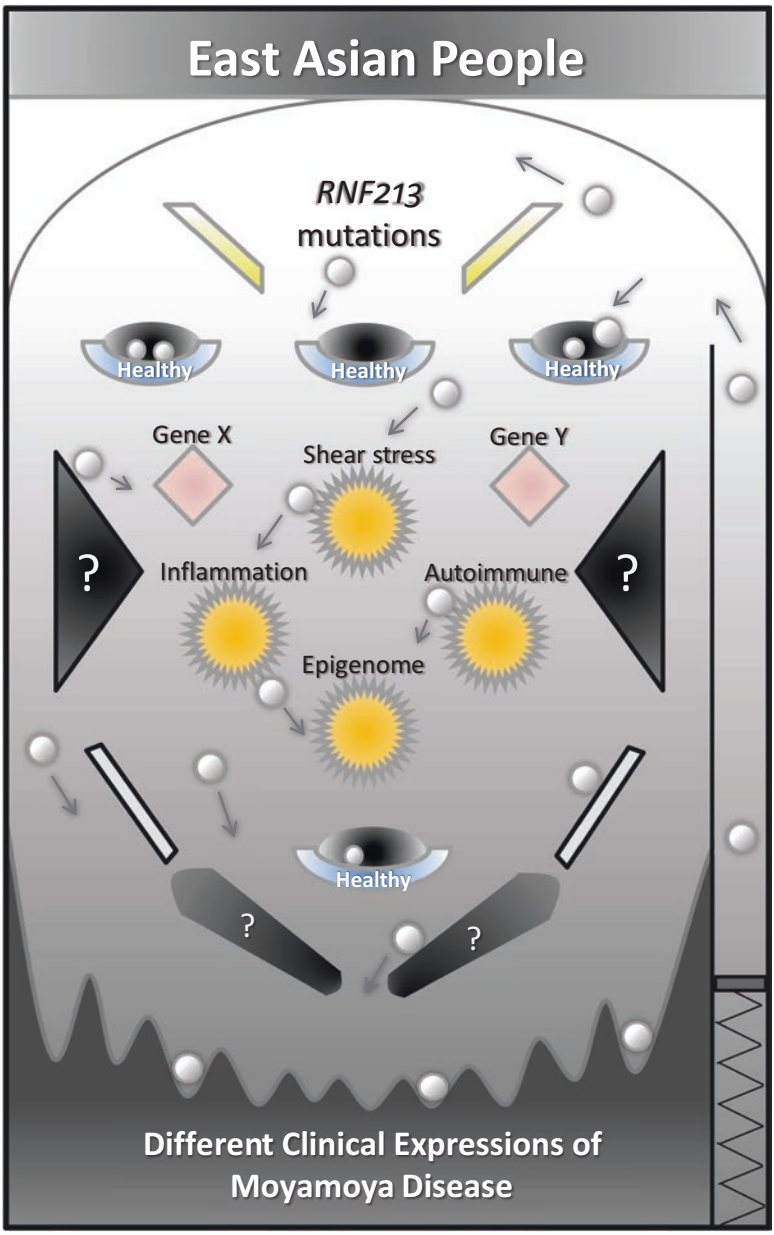

Fig. 2.1 Pinball model representing the development of MMD. Balls rolling down to different outcomes represent individual East Asian people. Most people are healthy even with *RNF213* mutations. *RNF213* mutation is a major susceptibility factor, but multiple other factors interact and contribute to the development and the different clinical expressions of MMD. The contributing factors likely vary according to race

References

1. Kossorotoff M. Cognitive decline in Moyamoya: influence of chronic cerebral hypoxia, history of stroke, or comorbid conditions? Dev Med Child Neurol. 2012;54(1):5–6. doi:10.1111/j.1469-8749.2011.04159.x.
2. Kazumata K, Tha KK, Narita H, Kusumi I, Shichinohe H, Ito M, Nakayama N, Houkin K. Chronic ischemia alters brain microstructural integrity and cognitive performance in adult Moyamoya disease. Stroke. 2015;46(2):354–60. doi:10.1161/STROKEAHA.114.007407.
3. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7(11):1056–66. doi:10.1016/S1474-4422(08)70240-0.
4. Kuroda S, Hashimoto N, Yoshimoto T, Iwasaki Y, Research Committee on Moyamoya Disease in J. Radiological findings, clinical course, and outcome in asymptomatic Moyamoya disease: results of multicenter survey in Japan. Stroke. 2007;38(5):1430–5. doi:10.1161/STROKEAHA.106.478297.
5. Research Committee on the Pathology, Treatment of Spontaneous Occlusion of the Circle of Willis, Health Labour Sciences Research Grant for Research on Measures for Infractable Diseases. Guidelines for diagnosis and treatment of Moyamoya disease (spontaneous occlusion of the circle of Willis). Neurol Med Chir (Tokyo). 2012;52(5):245–66.
6. Uchino H, Kim JH, Fujima N, Kazumata K, Ito M, Nakayama N, Kuroda S, Houkin K. Synergistic interactions between direct and indirect bypasses in combined procedures: the significance of indirect bypasses in Moyamoya disease. Neurosurgery. 2016; doi:10.1227/NEU.0000000000001201.
7. Takeuchi KSK. Hypoplasia of the bilateral internal carotid arteries. Brain Nerve. 1957; 9:37–43.
8. Suzuki J, Takaku A. Cerebrovascular "Moyamoya" disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20(3):288–99.
9. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, Nakatomi H, Shimizu T, Saito N. Genetic variant RNF213 c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2013;44(10):2894–7. doi:10.1161/STROKEAHA.113.002477.
10. Fujimura M, Tominaga T. Lessons learned from Moyamoya disease: outcome of direct/indirect revascularization surgery for 150 affected hemispheres. Neurol Med Chir (Tokyo). 2012;52(5):327–32.
11. Czabanka M, Pena-Tapia P, Schubert GA, Heppner FL, Martus P, Horn P, Schmiedek P, Vajkoczy P. Proposal for a new grading of Moyamoya disease in adult patients. Cerebrovasc Dis. 2011;32(1):41–50. doi:10.1159/000326077.
12. Houkin K, Ito M, Sugiyama T, Shichinohe H, Nakayama N, Kazumata K, Kuroda S. Review of past research and current concepts on the etiology of Moyamoya disease. Neurol Med Chir (Tokyo). 2012;52(5):267–77.
13. Phi JH, Wang KC, Lee JY, Kim SK. Moyamoya syndrome: A window of Moyamoya disease. J Korean Neurosurg Soc. 2015;57(6):408–14. doi:10.3340/jkns.2015.57.6.408.
14. Guey S, Tournier-Lasserve E, Herve D, Kossorotoff M. Moyamoya disease and syndromes: from genetics to clinical management. Appl Clin Genet. 2015;8:49–68. doi:10.2147/TACG.S42772.
15. Komiyama M, Nishikawa M, Yasui T, Otsuka M, Haze K. Moyamoya disease and coronary artery disease–case report. Neurol Med Chir (Tokyo). 2001;41(1):37–41.
16. Goto Y, Yonekawa Y. Worldwide distribution of Moyamoya disease. Neurol Med Chir (Tokyo). 1992;32(12):883–6.
17. Uchino K, Johnston SC, Becker KJ, Tirschwell DL. Moyamoya disease in Washington state and California. Neurology. 2005;65(6):956–8. doi:10.1212/01.wnl.0000176066.33797.82.
18. Yonekawa Y, Ogata N, Kaku Y, Taub E, Imhof HG. Moyamoya disease in Europe, past and present status. Clin Neurol Neurosurg. 1997;99(Suppl 2):S58–60.

19. Kainth D, Chaudhry SA, Kainth H, Suri FK, Qureshi AI. Epidemiological and clinical features of Moyamoya disease in the USA. Neuroepidemiology. 2013;40(4):282–7. doi:10.1159/000345957.
20. Kraemer M, Heienbrok W, Berlit P. Moyamoya disease in Europeans. Stroke. 2008;39(12):3193–200. doi:10.1161/STROKEAHA.107.513408.
21. Acker G, Goerdes S, Schneider UC, Schmiedek P, Czabanka M, Vajkoczy P. Distinct clinical and radiographic characteristics of Moyamoya disease amongst European Caucasians. Eur J Neurol. 2015;22(6):1012–7. doi:10.1111/ene.12702.
22. Fukui M, Kono S, Sueishi K, Ikezaki K. Moyamoya disease. Neuropathology. 2000;20(Suppl):S61–4.
23. Takekawa Y, Umezawa T, Ueno Y, Sawada T, Kobayashi M. Pathological and immunohistochemical findings of an autopsy case of adult Moyamoya disease. Neuropathology. 2004;24(3):236–42.
24. Masuda J, Ogata J, Yutani C. Smooth muscle cell proliferation and localization of macrophages and T cells in the occlusive intracranial major arteries in Moyamoya disease. Stroke. 1993;24(12):1960–7.
25. Kaku Y, Morioka M, Ohmori Y, Kawano T, Kai Y, Fukuoka H, Hirai T, Yamashita Y, Kuratsu J. Outer-diameter narrowing of the internal carotid and middle cerebral arteries in Moyamoya disease detected on 3D constructive interference in steady-state MR image: is arterial constrictive remodeling a major pathogenesis? Acta Neurochir. 2012;154(12):2151–7. doi:10.1007/s00701-012-1472-4.
26. Hosoda Y, Ikeda E, Hirose S. Histopathological studies on spontaneous occlusion of the circle of Willis (cerebrovascular Moyamoya disease). Clin Neurol Neurosurg. 1997;99(Suppl 2):S203–8.
27. Blecharz KG, Frey D, Schenkel T, Prinz V, Bedini G, Krug SM, Czabanka M, Wagner J, Fromm M, Bersano A, Vajkoczy P. Autocrine release of angiopoietin-2 mediates cerebrovascular disintegration in Moyamoya disease. J Cereb Blood Flow Metab. 2016; doi:10.1177/0271678X16658301.
28. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, Taura D, Sone M, Asaka I, Ameku T, Watanabe A, Kasahara T, Sudo T, Shiota F, Hashikata H, Takagi Y, Morito D, Miyamoto S, Nakao K, Koizumi A. 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(1):13–9. doi:10.1016/j.bbrc.2013.07.004.
29. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of Moyamoya disease and associated health risks explored through RNF213. Environ Health Prev Med. 2016;21(2):55–70. doi:10.1007/s12199-015-0498-7.
30. Kobayashi H, Matsuda Y, Hitomi T, Okuda H, Shioi H, Matsuda T, Imai H, Sone M, Taura D, Harada KH, Habu T, Takagi Y, Miyamoto S, Koizumi A. Biochemical and functional characterization of RNF213 (Mysterin) R4810K, a susceptibility mutation of Moyamoya disease, in angiogenesis in vitro and in vivo. J Am Heart Assoc. 2015;4(7) doi:10.1161/JAHA.115.002146.
31. Achrol AS, Guzman R, Lee M, Steinberg GK. Pathophysiology and genetic factors in Moyamoya disease. Neurosurg Focus. 2009;26(4):E4. doi:10.3171/2009.1.FOCUS08302.
32. Psaltis PJ, Simari RD. Vascular wall progenitor cells in health and disease. Circ Res. 2015;116(8):1392–412. doi:10.1161/CIRCRESAHA.116.305368.
33. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997;275(5302):964–7.
34. Kang HS, Moon YJ, Kim YY, Park WY, Park AK, Wang KC, Kim JE, Phi JH, Lee JY, Kim SK. Smooth-muscle progenitor cells isolated from patients with Moyamoya disease: novel experimental cell model. J Neurosurg. 2014;120(2):415–25. doi:10.3171/2013.9.JNS131000.
35. Sugiyama T, Kuroda S, Nakayama N, Tanaka S, Houkin K. Bone marrow-derived endothelial progenitor cells participate in the initiation of Moyamoya disease. Neurol Med Chir (Tokyo). 2011;51(11):767–73.

36. Hojo M, Hoshimaru M, Miyamoto S, Taki W, Nagata I, Asahi M, Matsuura N, Ishizaki R, Kikuchi H, Hashimoto N. Role of transforming growth factor-beta1 in the pathogenesis of Moyamoya disease. J Neurosurg. 1998;89(4):623–9. doi:10.3171/jns.1998.89.4.0623.
37. Houkin K, Yoshimoto T, Abe H, Nagashima K, Nagashima M, Takeda M, Isu T. Role of basic fibroblast growth factor in the pathogenesis of Moyamoya disease. Neurosurg Focus. 1998;5(5):e2.
38. Nanba R, Kuroda S, Ishikawa T, Houkin K, Iwasaki Y. Increased expression of hepatocyte growth factor in cerebrospinal fluid and intracranial artery in Moyamoya disease. Stroke. 2004;35(12):2837–42. doi:10.1161/01.STR.0000148237.13659.e6.
39. Fujimura M, Watanabe M, Narisawa A, Shimizu H, Tominaga T. Increased expression of serum Matrix Metalloproteinase-9 in patients with Moyamoya disease. Surg Neurol. 2009;72(5):476–80. discussion 480 doi:10.1016/j.surneu.2008.10.009.
40. Kang HS, Kim JH, Phi JH, Kim YY, Kim JE, Wang KC, Cho BK, Kim SK. Plasma matrix metalloproteinases, cytokines and angiogenic factors in Moyamoya disease. J Neurol Neurosurg Psychiatry. 2010;81(6):673–8. doi:10.1136/jnnp.2009.191817.
41. Bersano A, Guey S, Bedini G, Nava S, Herve D, Vajkoczy P, Tatlisumak T, Sareela M, van der Zwan A, Klijn CJ, Braun KP, Kronenburg A, Acerbi F, Brown MM, Calviere L, Cordonnier C, Henon H, Thines L, Khan N, Czabanka M, Kraemer M, Simister R, Prontera P, Tournier-Lasserve E, Parati E, European Moyamoya Disease I. Research progresses in understanding the pathophysiology of Moyamoya disease. Cerebrovasc Dis. 2016;41(3–4):105–18. doi:10.1159/000442298.
42. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, Kanno J, Niihori T, Ono M, Ishii N, Owada Y, Fujimura M, Mashimo Y, Suzuki Y, Hata A, Tsuchiya S, Tominaga T, Matsubara Y, Kure S. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40. doi:10.1038/jhg.2010.132.
43. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, Hashikata H, Matsuura N, Yamazaki S, Toyoda A, Kikuta K, Takagi Y, Harada KH, Fujiyama A, Herzig R, Krischek B, Zou L, Kim JE, Kitakaze M, Miyamoto S, Nagata K, Hashimoto N, Koizumi A. Identification of RNF213 as a susceptibility gene for Moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542.
44. Liu W, Senevirathna ST, Hitomi T, Kobayashi H, Roder C, Herzig R, Kraemer M, Voormolen MH, Cahova P, Krischek B, Koizumi A. Genomewide association study identifies no major founder variant in Caucasian Moyamoya disease. J Genet. 2013;92(3):605–9.
45. Ganesan V, Smith ER. Moyamoya: defining current knowledge gaps. Dev Med Child Neurol. 2015;57(9):786–7. doi:10.1111/dmcn.12708.
46. Bush AM, Borzage MT, Choi S, Vaclavu L, Tamrazi B, Nederveen AJ, Coates TD, Wood JC. Determinants of resting cerebral blood flow in sickle cell disease. Am J Hematol. 2016;91(9):912–7. doi:10.1002/ajh.24441.
47. Sensenbach W, Madison L, Eisenberg S, Ochs L. The cerebral circulation and metabolism in hyperthyroidism and myxedema. J Clin Invest. 1954;33(11):1434–40. doi:10.1172/JCI103021.
48. Dai D, Lu Q, Huang Q, Yang P, Hong B, Xu Y, Zhao W, Liu J, Li Q. Serum miRNA signature in Moyamoya disease. PLoS One. 2014;9(8):e102382. doi:10.1371/journal.pone.0102382.
49. Zhao S, Gong Z, Zhang J, Xu X, Liu P, Guan W, Jing L, Peng T, Teng J, Jia Y. Elevated Serum MicroRNA Let-7c in Moyamoya disease. J Stroke Cerebrovasc Dis. 2015;24(8):1709–14. doi:10.1016/j.jstrokecerebrovasdis.2015.01.041.
50. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007;128(4):635–8. doi:10.1016/j.cell.2007.02.006.

Part II
Genetic Epidemiology

Chapter 3
Molecular Epidemiology in East Asian Countries and in the World

Wanyang Liu

Abstract Although the etiology of moyamoya disease (MMD) remains unknown, recent genetic studies have identified *RNF213* p.R4810K in the 17q25-ter region as the most important founder susceptibility gene for MMD among East Asian populations, mainly including Japanese, Korean, and Chinese. Following the discovery studies, *RNF213* p.R4810K was replicated as an important founder susceptibility gene for MMD among many additional cohorts of East Asian populations. Moreover, many rare variants other than the founder one in *RNF213* also contributed to MMD across different populations in the world. Possibly because of the presence of the major founder effects of *RNF213* p.R4810K in East Asian patients, but not in Caucasian patients, the incidence and prevalence of MMD is relatively higher in East Asian countries than those in other countries. This chapter will discuss the molecular epidemiology of MMD in Asian and other populations in the world.

Keywords Moyamoya disease • Intracranial major artery stenosis/occlusion • *RNF213* • Molecular epidemiology • East Asian

Although moyamoya disease (MMD) etiology remains unknown, strong Asian ethnicity-related effect, a high disease concordance rate among monozygotic twins, and approximately 10–15% familial history of the patients with MMD strongly suggest the genetic components play an important role in the development of MMD. So far, five genetic loci have been identified to be linked to MMD in Japanese patients, including 3p24.2–p26, 6q25, 8q23, 12p12, and 17q25 [1–5]. Although the previously genetic results were conflicting and unrepeatable, a dozen of candidate genes or alleles have been reported to increase the susceptibility of MMD among the different ethnicities in the world [6–12]. Until recently, p.R4810K in *RNF213* located

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in 17q25.3-ter was identified as the first major founder susceptibility gene for MMD in East Asians, such as Japanese, Korean, and Chinese (Table 3.1) [13, 14]. Shortly after the discovery studies, p.R4810K, and other rare variants in *RNF213*, was collectively confirmed to be an important susceptibility gene among the different populations in the world by many research groups (Table 3.1) [15–32]. This chapter will mainly focus on the research findings of the genetic studies of *RNF213* of MMD among different populations in East Asia and in the world.

3.1 Discovery Stage: Identification of *RNF213*, the Most Important Founder Susceptibility Gene for MMD

In 2011, *RNF213* located in the previous linkage region of 17q25.3 was identified as the first important susceptibility gene for MMD by two Japanese research groups using the different approaches (Table 3.1) [13, 14]. Liu et al. performed a combined study of genome-wide linkage analysis and whole exome sequencing (WES) in eight Japanese familial MMD with at least three-generation affected members. Genome-wide linkage analysis revealed MMD linkage to 17q25.3 with a LOD score of 8.46 at D17S784. Fine mapping in the linkage region further narrowed the region to a 1.5-Mb disease locus bounded by D17S1806 and rs2280147, harboring 21 candidate genes. WES revealed p.R4810K in *RNF213* in the 1.5-Mb linkage region common to the eight index cases from eight families. Sequencing *RNF213* in 42 Japanese pedigrees with familial history for MMD confirmed p.R4810K to be segregated with disease perfectly. The SNPs genotyping around *RNF213* p.R4810K revealed a common haplotype transmitted in 42 families, which strongly suggested that *RNF213* p.R4810K was a founder susceptibility gene for MMD. A case-control association study across different ethnicities revealed that *RNF213* p.R4810K was significantly associated with sporadic MMD in East Asian populations (P=6.61×10^{-120}, OR=111.84, 95% CI=64.01–195.39), including Japanese (P=1.05×10^{-100}, OR=338.94, 95% CI=147.82–777.44), Korean (P=7.58×10^{-27}, OR=135.63, 95% CI=43.03–427.52), and Chinese (P=2.63×10^{-5}, OR=14.70, 95% CI=3.05–70.81). Five novel variants, p.D4863N, p.E4950D, p.A5021V, p.D5160E, and p.E5176G, were identified in non-p.R4810K East Asian patients. The p.R4810K and its founder haplotype were not identified among Caucasian cases; however, four rare variants p.N3962D, p.D4013N, p.R4062Q, and p.P4608S were identified (Table 3.1) [13]. This East Asian specific founder susceptibility gene of *RNF213* p.R4810K may be responsible for the high prevalence of MMD among East Asians and its low prevalence among Caucasians. The identification of MMD susceptibility gene in this study indicates that the combined WES with linkage analysis is useful to identify novel susceptibility loci to human diseases or traits.

At the same time, Kamada et al. performed a genome-wide association study (GWAS) to identify MMD susceptibility gene in a Japanese cohort comparing 72 MMD patients and 45 controls. A genome-wide significant association locus was

Table 3.1 Genetic studies on MMD susceptibility genes *RNF213*

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Liu et al. 2011 [13]	Japanese: (FMMD: 42, SMMD: 161/384), Korean (38/223), Chinese (52/150), Caucasian (50/384)	Genome-wide linkage analysis	Linkage to 17q25.3	*RNF213* was first major founder susceptibility gene for East Asian MMD, including Japanese, Korean, and Chinese
		WES to identify rare functional variants	Segregation with the *RNF213* p.R4810K and its founder haplotype	Rare variants in *RNF213* also confer the susceptibility for Caucasian and East Asian MMD without p.R4810K
		Association study for *RNF213* p.R4810K according to ethnicity	Strong association with the p.R4810K for East Asian sporadic MMD, but not for Caucasians	
			Rare *RNF213* variants identified in Caucasian and East Asian patients without p.R4810K	
Kamada et al. 2011 [14]	Japanese (72/45)	Linkage analysis focused on the previous candidate loci	Association of MMD with locus 17q25-ter	*RNF213* was identified as first major founder susceptibility gene for Japanese MMD
		GWAS and locus-specific association study	Strong association with the *RNF213* locus	Rare variants in *RNF213* also confer the susceptibility for Japanese MMD
		Association study for *RNF213* p.R4810K	Strong association with the p.R4810K in *RNF213*	
			Rare *RNF213* variants identified in Japanese patients without p.R4810K	

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Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Miyatake et al. 2012 [15]	Japanese (204/283)	Association study for *RNF213* p.R4810K	*RNF213* p.R4810K found in 95.1% of familial MMD cases, in 79.2% of sporadic cases, and in 1.8% of controls (OR = 259, 95% CI= 100–674, $P<0.001$)	*RNF213* was a major susceptibility gene for Japanese familial and sporadic MMD
		Analysis of genotype-phenotype correlation	Patients with homozygous p.R4810K present with an earlier onset, an increased risk of infarction at initial presentation, and an increased rate of PCA involvement	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
Miyatake et al. 2012 [16]	Japanese (Sibling cases)	Case-control study for *RNF213* p.R4810K	Earlier onset and more severe course in patients with homozygous p.R4810K	*RNF213* p.R4810K was a good biomarker on the progression and prognosis of disease
		Analysis of genotype-phenotype correlation		
Miyawaki et al. 2012 [17]	Japanese (48 MMD, 41 non-MMD ICASO)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 85.4% of MMD patients, in 21.9% of non-MMD ICASO patients, in 1.6% of cerebral aneurysm patients, in no cervical disease patients, and in no controls	*RNF213* p.R4810K was a major susceptibility gene not only for MMD but also for non-MMD ICASO with Japanese ethnicity
		Analysis of genotype-phenotype correlation	Significant associations of p.R4810K with MMD (OR= 292.8, 95% CI= 15.4–5153, $P<0.0001$) and non-MMD ICASO (OR= 14.9, 95% CI= 0.82–268.4, $P=0.01$); no association with either cerebral aneurysm or cervical disease	

Miyawaki et al. 2013 [18]	Japanese (323/110)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 16/22 of the definite MMD patients, in 4/8 of the unilateral MMD patients, in 20/84 of non-MMD ICASO patients, in 1/34 of extracranial carotid atherosclerosis patients, in no patients with cerebral aneurysm and intracerebral hemorrhage, in 1.8% of controls	*RNF213* p.R4810K was a major susceptibility gene not only for MMD but also for non-MMD ICASO with Japanese ethnicity
		Analysis of genotype-phenotype correlation	Significant associations of p.R4810K with definite MMD (OR= 144.0, 95% CI= 26.7–775.9, $P<0.0001$), unilateral MMD (OR= 54.0, 95% CI= 7.5–386.8, $P=0.0001$), and non-MMD ICASO (OR= 16.8, 95% CI= 3.81–74.5, $P<0.0001$); no association with extracranial carotid atherosclerosis, cerebral aneurysm, and intracerebral hemorrhage	
Chung et al. 2016 [19]	Korean (146 cases only)	Association study for the *RNF213* p.R4810K with basal collaterals	*RNF213* p.R4810K observed in 65.8% of adult-onset MMD patients and not associated with the degree of basal collaterals ($P = 0.289$)	*RNF213* p.R4810K was a major susceptibility gene for Korean adult-onset MMD
Jang et al. 2015 [21]	Korean (1516 controls only)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 2.44% of controls	*RNF213* p.R4810K was a major susceptibility gene for Korean MMD
			Significant association of p.R4810K with MMD ($P < 0.001$) (OR=162.7, 95% CI=65.5–403.9; OR=137.8, 95% CI=55.8–339.9, based on the cord blood and adults samples, respectively)	

(continued)

Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Kim et al. 2016 [22]	Korean (165/294)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 75.8% of MMD patients and in 2.72% of controls. Significant association of p.R4810K with MMD ($P<0.001$)	*RNF213* p.R4810K was a major susceptibility gene for Korean MMD
		Analysis of genotype-phenotype correlation	Significant associations of homozygous p.R4810K with early-onset MMD, cerebral infarction at diagnosis, and cognitive impairment in long-term outcome	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
Bang et al. 2016 [23]	Korean (288 with MMD, 234 with ICASO)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K observed in 69.1% MMD patients, 21.4% ICASO patients	*RNF213* is a susceptibility gene, not only for MMD but also for ICASO in Korean
		Analysis of genotype-phenotype correlation	Among ICASO patients, p.R4810K carriers were younger and more likely to have a family MMD history than noncarriers were	
			Independently association of the age of ICASO onset with the p.R4810K (OR =0.97, 95% CI =0.944–0.99)	

Wu et al. 2012 [24]	Chinese Han (170/507)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 13% of the patients, 0.4% of the controls	*RNF213* variants were the major susceptibility gene for Chinese MMD
		Analysis of genotype-phenotype correlation	Significant association of p.R4810K with MMD (P=6.1×10^{-15}). p.R4810K more frequently found in ischemic versus hemorrhagic MMD (P = 0.001)	*RNF213* p.R4810K was a good biomarker for the progression and prognosis of disease
		Mutational sequencing covering *RNF213* exon 40 to exon 68	Eight rare variants identified, p.A4399T found in 16.5% of cases and 8.9% of controls and associated with MMD (OR=2.0, 95% CI=1.2–3.3, P=0.004), especially with hemorrhage (OR =2.8, 95% CI=1.2–6.5, P=0.014)	
Wang et al. 2013 [25]	Chinese Han (96/96)	Association study for candidate polymorphisms in the *PDFRBR/MMP3/TIMP2/RNF213/Raptor* genes and searching for gene-gene interactions	Association of MMD with *RNF213* p.R4810K and rs148731719	*RNF213* p.R4810K and rs148731719 may independently exert a significant influence on MMD occurrence
			No significant gene-gene interaction detected	
Shang et al. 2015 [26]	Chinese Han (139 ICASO, 146 non-ICASO/300)	Association study for the *RNF213* p.R4810K	*RNF213* p.R4810K found in 0.35% of ischemic stroke patients, in 0.72% of ICASO patients, none in non-ICASO subgroup, and 0.33% of the controls	*RNF213* p.R4810K may be not associated with ischemic stroke and ICASO in Chinese Han population
		Analysis of genotype-phenotype correlation	No associations of p.R4810K with ischemic stroke and ICASO	

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Table 3.1 (continued)

Author, year	Ethnicity (sample size: case/control)	Study approach	Results: identified loci/markers	Conclusions: significances/ implications
Huang et al. 2015 [27]	Chinese Guangxi Zhuang (52 MMD, 64 ICASO/80)	Association study for the *RNF213* rs138130613,p.R4810K and rs148731719 polymorphism	Significant associations of *RNF213* p.R4810K with both patients with MMD (P=0.006) and non-MMD ICASO (P=0.045), but no associations for rs138130613 and rs148731719 in *RNF213*	*RNF213* p.R4810K might be a genetic marker for MMD and might be related to the formation of intracranial major artery stenosis/occlusion in Guangxi Zhuang population
		Analysis of genotype-phenotype correlation		
Zhang et al. 2016 [28]	Chinese (255/300)	Association study for the *RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3* polymorphism	Twenty-seven rare variants of *RNF213* identified in MMD and not found in controls. Among them, p.R4810K identified in 31.4% of patients (P<0.000). Twenty-five rare variants identified in 10.6% of patients without p.R4810K variants. No possible disease variants identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with patients without the *RNF213* rare variants, the p.R4810K heterozygous patients were younger at diagnosis (25 vs 29 years old, P=0.049) and had more familial cases (24% vs 4.4%, P=0.000), ischemic cases (81.3% vs 67.5%, P=0.037), and involvement of the PCA (52% vs 32.5%, P=0.007)	*RNF213* is the major susceptibility gene for Chinese MMD patients
		Analysis of genotype-phenotype correlation		The p.R4810K heterozygous patients exhibited different clinical features, compared to patients without the rare variants in *RNF213*
Lee et al. 2015 [29]	Taiwanese (36/500)	Mutational sequencing covering all exons in *RNF213*	Four different *RNF213* variants, p. R4810K, p.A1622V, p.V3933 M, and p. R4131C, identified in 11 of 36 patients	*RNF213* was a major susceptibility gene for Taiwanese MMD
		Association study for the *RNF213* p.R4810K		

Liu et al. 2013 [30]	Central European (38/41)	GWAS	No significant association with polymorphisms in *RNF213* and *ACTA2* genes	No major founder variant in Caucasian MMD was found, although several suggestive association regions were identified
		Sequencing of candidate genes in suggestive association regions	Suggestive associations with four loci	
Cecchi et al. 2014 [31]	Asian descent (16/−), non-Asian descent (94/−)	Mutational sequencing in 86 patients covering *RNF213* exon 43–45 and exon 60	*RNF213* p.R4810K found in 56% of MMD patients of Asian descent, in no of 94 patients of non-Asian descent	The variants in *RNF213* predispose patients of diverse ethnicities to MMD. The p.R4810K variant predisposes individuals of Asian descent in the United States to MMD
		WES in 24 additional patients covering the entire *RNF213* exons	Rare variants identified by targeted exon sequencing and WES	
Shoemaker et al. 2016 [32]	Asian descent and non-Asian descent (125/125)	WES to further develop variant landscape of MMD in multiethnic population	*RNF213* p.R4810K enriched among East Asians (P=6.01×10^{-5}) and none in Caucasian cases and controls. The most enriched variant in Caucasian (P=7.93×10^{-4}) and non-*RNF213* founder mutation (P=1.51×10^{-3}) cases was *ZXDC* p.P562L, a gene involved in MHC class II activation, and *OBSCN*, a gene involved in myofibrillogenesis	*RNF213* p.R4810K was the East Asian origins.
				Novel, alternative candidate variants and genes in addition to *RNF213* may be important in multiethnic MMD etiology and diagnosis

Abbreviations: *MMD* moyamoya disease, *FMMD* familial MMD, *SMMD* sporadic MMD, *WES* whole exome sequencing, *GWAS* genome-wide association study, *OR* odds ratio, *CI* confidence interval, *PCA* posterior cerebral artery, *ICASO* intracranial major artery stenosis/occlusion

found at 17q25-ter with Japanese MMD risk ($P<10^{-8}$). A locus-specific association study in the 17q25-ter further confirmed the genome-wide association locus and identified a haplotype at the *RNF213* locus significantly associated with MMD risk ($P=5.3\times10^{-10}$). Sequencing of *RNF213* revealed a founder variant p.R4810K, in 95% of MMD families, 73% of non-familial MMD cases, and 1.4% of controls among Japanese population. The p.R4810K dramatically increased the risk of MMD with an odds ratio of 190.8 ($P=1.2\times10^{-43}$, 95% CI=71.7–507.9). Three additional missense variants, p. M3891V, p.V4567M, and p.V4765M, were detected in three non-p.R4810K sporadic patients (Table 3.1) [14]. These two discovery studies successfully identified the same susceptibility genes of *RNF213* p.R4810K for MMD in various East Asian ethnicities using the different approaches, which suggest MMD with different ethnicities may share common major susceptibility genes and disease etiology. Further genetic studies are needed to validate this hypothesis in the different ethnicities with a large number of sample sizes.

3.2 Replication Stage: Replication of *RNF213* as a Susceptibility Gene for MMD Across Different Ethnicities

3.2.1 Japanese MMD

Shortly after the discovery studies, *RNF213* has been subsequently confirmed to be a major susceptibility gene in many additional studies across different ethnicities, including Japanese, Korean, Chinese, Europeans, and American (Table 3.1) [13–32]. Miyatake et al. sequenced the entire coding regions of the *RNF213* gene in 204 patients with MMD and evaluate the risk of corresponding variants detected in the parents of the patients and 283 controls. The p.R4810K variant was confirmed to be strongly associated with MMD in this additional Japanese cohort ($P<0.001$, OR=259, 95% CI=100–674). The p.R4810K was identified in 95.1% of familial MMD cases, 79.2% of sporadic cases, and 1.8% of normal controls. Moreover, the correlations of genotype-phenotype associated with gene dosage were also observed. Compared with heterozygotes or wild types, homozygotes of p.R4810K had a significantly earlier age at onset, significantly higher frequencies of infarctions at initial presentation, and involvement of posterior cerebral arteries (PCAs) than those in heterozygotes and wild types. Eighteen other rare variants were also identified in the patients without p. R4810K and were not associated with any clinical phenotypes of MMD (Table 3.1) [15]. Followed by their first study on the gene dosage effects of the *RNF213* variant, *RNF213* p.R4810K also showed the gene doses effects in Japanese sibling cases with MMD. In this study, *RNF213* p.R4810K showed a significant association with MMD, as well as different clinical course and disease severity. The patients with the homozygote of p.R4810K in the sibling pair showed an early-onset age and rapid disease progress, compared with those with the

heterozygote of the variant (Table 3.1) [16]. These two studies suggest an obvious correlation of genotype-phenotype of *RNF213* p.R4810K with MMD.

Additional two studies reported by the same research group replicated the previous association of the *RNF213* p.R4810K with Japanese MMD risk (Table 3.1) [17, 18]. The first study evaluated the frequencies of p.R4810K variant in 48 patients with MMD, as well as in 41 patients with intracranial major artery stenosis/occlusion (ICASO) without signs of MMD (non-MMD ICASO). The 85.4% patients with MMD and 21.9% patients with non-MMD ICASO had the risk allele of p.R4810K. The p.R4810K showed significant associations with MMD (P<0.0001, OR=292.8, 95% CI=15.4–5153) and with non-MMD ICASO (P=0.01, OR=14.9, 95% CI=0.82–268.4), but no association with either cerebral aneurysm or cervical disease. This study, for the first time, suggests the p.R4810K variant in *RNF213* common to both MMD and non-MMD ICASO (Table 3.1) [17]. To confirm the reality of the association of p.R4810K variant with non-MMD ICASO, Miyawaki et al. investigated the frequencies of p.R4810K variant in 323 patients with various phenotypes of intracranial major artery stenosis/occlusion and 110 control subjects in a different larger Japanese cohort from the previous one. Sixteen of 22 patients with definite MMD, 4/8 patients with unilateral MMD, 20/84 patients with non-MMD ICASO, 1/34 patients with extracranial carotid atherosclerosis, 0/44 patients with cerebral aneurysm, 0/21 patients with intracerebral hemorrhage, and 1.8% controls had the risk allele of p.R4810K. *RNF213* p.R4810K showed significant associations with definite MMD (P<0.0001, OR=144.0, 95% CI=26.7–775.9), unilateral MMD (P=0.0001, OR=54.0, 95% CI=7.5–386.8), and non-MMD ICASO (P<0.0001, OR=16.8, 95% CI=3.81–74.5), but no associations with extracranial carotid atherosclerosis, cerebral aneurysm, or intracerebral hemorrhage (Table 3.1) [18]. The authors replicated the previous findings and proposed the existence of a new entity of ICASO caused by the p.R4810K variant in *RNF213* at least for Japanese ethnicity.

3.2.2 Korean MMD

Following on from the discovery studies [13, 14], several studies have replicated the association of *RNF213* p.R4810K with MMD risk in Korean population (Table 3.1) [19–23]. Chung et al. evaluated the association between clinical, genetic, and radiologic factors and basal collaterals in 146 Korean patients with MMD. The *RNF213* p.R4810K was observed in 50 (65.8%) adult-onset MMD patients and not associated with the degree of basal collaterals (P=0.289) (Table 3.1) [19]. Actually, this study did not evaluate the association between the p.R4810K and MMD risk, due to a lack of allele frequency information in the controls. However, the significant association should exist in this Korean cohort based on the frequency data from other studies (Table 3.1) [12, 13, 20, 21].

Another study investigated the *RNF213* p.R4810K genotype and genotype-phenotype correlations in 165 Korean MMD patients and 294 controls by direct

sequencing of the major *RNF213* SNPs. The p.R4810K was detected in 75.8% of MMD patients and in 2.72% of controls, respectively. The p.R4810K significantly associated with the risk of Korean MMD, with an OR of 52.11 ($P<0.001$) compared with controls. Moreover, p.R4810K risk genotypes occurred more frequently in familial MMD patients than in sporadic patients. The homozygous p.R4810K showed a significant association with early age at onset, cerebral infarction at initial diagnosis, and cognitive impairment in long-term outcome (Table 3.1) [22]. The findings indicate that the p.R4810K risk allele is strongly associated with Korean patients with MMD and homozygous p.R4810K may be a good biomarker for early-onset MMD or unstable MMD with cerebral infarction, which requires early diagnosis and revascularization treatment.

Due to the high prevalence of both intracranial atherosclerotic stenosis (ICAS) and MMD in Asians, Bang, et al. hypothesized that the *RNF213* p.R4810K is also a susceptibility gene for the Korean patients with ICAS. The participants included 234 patients with ICAS and 288 with MMD. The *RNF213* p.R4810K was observed in 21.4% patients with ICAS and in 69.1% patients with MMD. ICAS patients with *RNF213* p.R4810K were younger and more likely to have a family MMD history than the patients without the variant were. Multivariate analysis revealed that only the age of ICAS onset was independently associated with the *RNF213* p.R4810K (OR=0.97, 95% CI=0.944–0.99) (Table 3.1) [23]. This study further demonstrated that *RNF213* is a susceptibility gene not only for MMD but also for ICAS in East Asians. Further studies on the association of *RNF213* variants in ICAS patients outside East Asian populations are needed.

3.2.3 Chinese MMD

After the identification of the founder variant p.R4810K and other rare patient-specific variants in *RNF213* as the important susceptibility genes for Chinese MMD (Table 3.1) [13], several genetic studies have also perfectly replicated the association of *RNF213* variants with MMD in Chinese population (Table 3.1) [24–29]. Wu et al. performed the first replication study on molecular analysis of *RNF213* in 170 MMD patients and 507 controls from Chinese Han population. The p.R4810K was detected in 13% of cases with MMD and 0.4% of 507 normal controls, respectively. The association of p.R4810K was perfectly replicated to greatly increase the risk for Chinese Han MMD ($P=6.1\times10^{-15}$, OR=36.7, 95% CI=8.6–156.6). The allele frequency of R4810K was significantly higher in ischemic versus hemorrhagic MMD ($P=0.001$, OR=5.4, 95% CI=1.8–16.1). Genomic sequencing covering later part of *RNF213* also identified eight other non-p.R4810K variants: p.P4007R, p.Q4367L, p.A4399T, p.T4586P, p.L4631V, p.E4950D, p.A5021V, and p.M5136I. Among them, p.A4399T variant was found in 16.5% of cases and 8.9% of controls and was significantly associated with MMD ($P=0.004$, OR=2.0, 95% CI=1.2–3.3), especially with hemorrhage ($P=0.014$, OR=2.8, 95% CI=1.2–6.5) (Table 3.1) [24].

This study validated the association of p.R4810K and expanded the spectrum of *RNF213* mutations in Chinese Han MMD.

Wang et al. evaluated the contributions and interactions of the polymorphisms of *RNF213* and other previously associated genes in 96 Chinese Han cases and 96 controls. Again, p.R4810K in *RNF213* showed a significant association with Chinese Han MMD (8.33%) compared with controls (1.04%) (*P*=0.018, OR=8.74). The significant association of the polymorphism rs148731719 in *RNF213* was also identified, but no association between MMD and other three loci in the genes of *PDGFRB*, *MMP-3*, and *TIMP-2*. There was no any significant interaction among these five loci by multifactor dimensionality reduction analysis. This study indicated that *RNF213* p.R4810K and rs148731719 may exert a significant influence on MMD occurrence than other genes in Chinese Han population (Table 3.1) [25]. Further studies in a larger sample size across different ethnicities are necessary to validate these contributions and interactions.

RNF213 p.R4810K has been associated with non-MMD ICASO patients in Japanese population (Table 3.1) [17, 18], but a lack of data in other populations. To validate the previous findings, Shang et al. explored the association between *RNF213* p.R4810K and ischemic stroke in a Chinese Han population with 285 patients and 300 controls. The patients with ischemic stroke were divided into ICASO subgroup (139) and non-ICASO subgroup (146). One of 285 patients with ischemic stroke, one of 139 patients with ICASO, none in non-ICASO subgroup, and one of 300 controls had the p.R4810K risk variant. Compared with controls, p.R4810K variant had no associations with ischemic stroke (*P*=1, OR=1.053, 95%CI=0.066–16.912) and ICASO (*P*=0.533, OR=2.167, 95%CI=0.135–34.894), respectively. This study failed to replicate the associations previously reported in Japanese patients with ICASO, indicating that *RNF213* p.R4810K variant may be not associated with ICASO in Chinese Han population (Table 3.1) [26]. Further studies are needed to validate the association between p.R4810K and ICASO phenotype in different ethnicities. Huang et al. investigated the association between rs138130613, p.R4810K, and rs148731719 variants of *RNF213* and the genetic susceptibility of adult MMD in a Guangxi Zhuang population. The participants consisted of 52 consecutive adult patients with MMD, 64 non-MMD ICASO, and 80 gender- and age-matched healthy controls. Compared with the control group, *RNF213* p.R4810K was found to be significantly associated with both MMD group and non-MMD ICASO group (*P*=0.006, OR=12.29, 95% CI=1.47–103.10; *P*=0.045, OR=8.17, 95% CI=0.96–69.74, respectively), but no associations for rs138130613 and rs148731719 in *RNF213*. These findings suggest that *RNF213* p.R4810K might be a genetic marker for MMD and might be related to the formation of ICASO in Guangxi Zhuang population (Table 3.1) [27]. Interestingly, the frequency difference in *RNF213* p.R4810K between Han [26] and Guangxi Zhuang populations with non-MMD ICASO indicates that the genetic background may be different even in different ethnic groups among Chinese population. Further genetic studies are needed to clarify the genetic etiology among different Chinese nationalities.

Zhang et al. performed a genetic study to identify disease-causing mutations in MMD association genes, including *RNF213*, *ACTA2*, *BRCC3*, and *GUCY1A3* in 255 Chinese MMD patients and 300 controls. Twenty-seven rare missense variants of *RNF213* were identified. Among them, p.R4810K was identified in 31.4% (80 of 255) of patients with MMD. Significantly higher frequencies of the p.R4810K in MMD patients were observed compared with controls (χ^2=104.166, P<0.000). Twenty-five rare variants were identified in 10.6% (27 of 255) of non-p.R4810K patients. The frequencies of rare and founder variants identified in this study (42%) were relatively higher than those of the previous studies (10–20%). In contrast, no disease-causing variants were identified in *ACTA2*, *BRCC3*, or *GUCY1A3*. Compared with patients without the rare *RNF213* variants, the patients with p.R4810K were younger at diagnosis (25 vs 29 years old, P=0.049) and had more familial cases (24% vs 4.4%, P=0.000), ischemic cases (81.3% vs 67.5%, P=0.037), and involvement of the PCA (52% vs 32.5%, P=0.007) (Table 3.1) [28]. Although the total proportion of *RNF213* variants was relatively lower than that in Japanese and Korean, *RNF213* is the major susceptibility gene in Chinese MMD patients. Compared to patients without the rare *RNF213* variants, the patients with p.R4810K showed relatively severe clinical features of MMD. One study sequenced all coding exons of *RNF213* in 36 Taiwanese MMD patients. Four different *RNF213* variants, p.R4810K, p.A1622V, p.V3933M, and p.R4131C, were identified in 11 patients (30.6%) (Table 3.1) [29]. This study replicated the previous association data and was the first genetic epidemiological study for MMD in Taiwan.

3.2.4 Caucasian MMD

Although several *RNF213* rare variants have been identified in Caucasian MMD [13], the mutation spectrum of MMD in Caucasian population still remains largely unknown. Liu et al. performed a GWAS in 38 cases and 41 controls to identify whether there is a major founder susceptibility gene for Caucasian MMD. This study failed to identify any major founder variant in Caucasian MMD as it is in East Asian MMD. However, several suggestive association regions were identified for Caucasian MMD (Table 3.1) [30].

More recently, two research groups independently investigated disease variants in multiethnic populations of MMD patients based in the United States by target exons sequencing and WES. These two genetic studies consistently replicated previous results and identified many novel rare variants in *RNF213* and other genes (Table 3.1) [31, 32]. Cecchi et al. investigated the contribution of *RNF213* variants to MMD among an ethnicities diverse population in the United States using targeted exon sequencing of *RNF213* or WES. The East Asian founder variant *RNF213* p.R4810K was found in 56% (9/16) of MMD patients of Asian descent, but not in 94 patients of the European, Hispanic, or African descent. Among nine patients of Asian descent carrying *RNF213* p.R4810K, seven were East Asian origin as previously reported; other were in novel groups not previously reported, including

Bangladeshi, Indian, and Filipino origin. Four rare *RNF213* variants were identified by Sanger sequencing: p.C3997Y, p.D4013N, p.R4019C, and p.I4076V. Seven additional rare variants were also identified in 29% (7/24) via WES: p.A529del, p.R3922Q, p.K4115del, p.D4237E, p.K4732T, p.E4950_F4951ins7, and p.V5163I. This study further confirms that rare genetic variants in *RNF213* predispose patients of diverse ethnicities to MMD and that the p.R4810K founder variant predisposes patients of Asian descent in the United States to MMD (Table 3.1) [31]. To further search the mutations for MMD, Shoemaker et al. conducted WES of 125 unrelated ethnically diverse MMD patients and 125 matched controls. According to ethnicity of the studied subjects, they established three subpopulations: Asian, Caucasian, and non-p.R4810K case. This study replicated the previously identified *RNF213* p.R4810K founder variant in Asian cases (P=6.01×10^{-5}) that was enriched among East Asians compared to Southeast Asian and Pacific Islander cases (P=9.52×10^{-4}) and was completely absent in all Caucasian cases and controls. Among Caucasian and non-p.R4810K cases, two most enriched variants were identified: *ZXDC* (p.P562L), a gene involved in MHC class II activation, and *OBSCN*, a gene involved in myofibrillogenesis. This study provides additional evidence on the East Asian origins of the *RNF213* p.R4810K variant and revealed novel, alternative candidate variants and genes that may be important in the etiology of MMD with multiethnicities (Table 3.1) [32].

3.3 Significance and Future Perspectives of *RNF213* on MMD

To date, many loci and genes were identified to be associated with the risk of MMD; however, the results were completely unrepeatable in different studies. In contrast, *RNF213* was confirmed to be the most important founder susceptibility gene for MMD with various ethnicities by many research groups in the world. The carrier frequencies of p.R4810K in East Asia were about 1–3% for controls and 20–80% for cases [13–29], but none in Caucasian cases and controls (Table 3.1) [30–32]. The previous data strongly suggest that the genetic background of MMD in East Asian is distinct from that in Westerners and the high incidence of MMD in East Asian countries may be attributable to the major founder effect of *RNF213*.

The homozygotes of *RNF213* p.R4810K had a significantly earlier-onset age, higher frequency of cerebral infarctions at diagnosis, and involvement of PCAs compared with heterozygotes or wild types, which strongly suggest that the homozygous *RNF213* p.R4810K could be a good biomarker for predicting the severe type, progression, and prognosis of MMD, for which early clinical intervention is recommended (Table 3.1) [15, 16, 22, 24, 28]. In addition, *RNF213* p.R4810K was significantly associated with not only MMD but also non-MMD ICASO in East Asian (Table 3.1) [17, 18, 23, 27], suggesting *RNF213* may be also important in the occurrence of ICASO phenotypes.

However, several questions remain and need to be elucidated. First, the large gap between the prevalence of carriers of the p.R4810K (1–3%) and the prevalence of MMD (1 in 10,000) strongly suggests that p.R4810K is a susceptibility factor to MMD, with an involvement of other unknown factors in the moyamoya phenotype. Further studies will need to figure out the mysterious other factors involved in MMD etiology in addition to *RNF213*. Secondly, the frequency of *RNF213* variants is relatively lower in Chinese MMD patients, compared to that in Japanese and Korean patients. The p.R4810K and other rare variants in *RNF213* could only explain about 20% cases in Chinese population. The major novel susceptibility genes for Chinese, as well as Caucasian MMD, remain to be discovered by next-generation sequencing tools in a large number of study populations.

References

1. Ikeda H, Sasaki T, Yoshimoto T, Fukui M, Arinami T. Mapping of a familial Moyamoya disease gene to chromosome 3p24.2-p26. Am J Hum Genet. 1999;64:533–7.
2. Inoue TK, Ikezaki K, Sasazuki T, Matsushima T, Fukui M. Linkage analysis of Moyamoya disease on chromosome 6. J Child Neurol. 2000;15:179–82.
3. Sakurai K, Horiuchi Y, Ikeda H, Ikezaki K, Yoshimoto T, Fukui M, et al. A novel susceptibility locus for Moyamoya disease on chromosome 8q23. J Hum Genet. 2004;49:278–81.
4. Yamauchi T, Tada M, Houkin K, Tanaka T, Nakamura Y, Kuroda S, et al. Linkage of familial Moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. Stroke. 2000;31:930–5.
5. Mineharu Y, Liu W, Inoue K, Matsuura N, Inoue S, Takenaka K, et al. Autosomal dominant Moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70(24 Pt 2):2357–63.
6. Kang HS, Kim SK, Cho BK, Kim YY, Hwang YS, Wang KC. Single nucleotide polymorphisms of tissue inhibitor of metalloproteinase genes in familial Moyamoya disease. Neurosurgery. 2006;58(6):1074–80.
7. Roder C, Peters V, Kasuya H, Nishizawa T, Takehara Y, Berg D, et al. Common genetic polymorphisms in Moyamoya and atherosclerotic disease in Europeans. Childs Nerv Syst. 2011;27(2):245–52.
8. Guo DC, Papke CL, Tran-Fadulu V, Regalado ES, Avidan N, Johnson RJ, 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(5):617–27.
9. Shimojima K, Yamamoto T. ACTA2 is not a major disease-causing gene for Moyamoya disease. J Hum Genet. 2009;54(11):687–8.
10. Roder C, Peters V, Kasuya H, Nishizawa T, Wakita S, Berg D, et al. Analysis of ACTA2 in European Moyamoya disease patients. Eur J Paediatr Neurol. 2011;15(2):117–22.
11. Hu FY, Zheng HB, Xu YM, Jiang Y, Zhou D. ACTA2 is not a major genetic risk gene for Chinese patients with Moyamoya disease. Int J Stroke. 2013;8(7):E43.
12. Liu W, Hashikata H, Inoue K, Matsuura N, Mineharu Y, Kobayashi H, et al. A rare Asian founder polymorphism of Raptor may explain the high prevalence of Moyamoya disease among East Asians and its low prevalence among Caucasians. Environ Health Prev Med. 2010;15:94–104.
13. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of *RNF213* as a susceptibility gene for Moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542.

14. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies *RNF213* as the first Moyamoya disease gene. J Hum Genet. 2011;56:34–40.
15. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576G>A variant of *RNF213* predicts early-onset and severe form of Moyamoya disease. Neurology. 2012;78:803–10.
16. Miyatake S, Touho H, Miyake N, Ohba C, Doi H, Saitsu H, et al. Sibling cases of Moyamoya disease having homozygous and heterozygous c.14576G>A variant in *RNF213* showed varying clinical course and severity. J Hum Genet. 2012;57:804–6.
17. Miyawaki S, Imai H, Takayanagi S, Mukasa A, Nakatomi H, Saito N. Identification of a genetic variant common to Moyamoya disease and intracranial major artery stenosis/occlusion. Stroke. 2012;43:3371–4.
18. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, et al. Genetic variant *RNF213* c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2013;44:2894–7.
19. Chung JW, Kim SJ, Bang OY, Kim KH, Ki CS, Jeon P, et al. Determinants of basal collaterals in Moyamoya disease: clinical and genetic factors. Eur Neurol. 2016;75:178–85.
20. Liu W, Harada KH, Hitomi T, Kobayashi H, Koizumi A. Distribution of Moyamoya disease susceptibility polymorphism p.R4810K in *RNF213* in East and Southeast Asian populations. Neurol Med Chir (Tokyo). 2012;52(5):299–303.
21. Jang MA, Shin S, Yoon JH, Ki CS. Frequency of the Moyamoya-related *RNF213* p.Arg4810Lys variant in 1,516 Korean individuals. BMC Med Genet. 2015;16:109.
22. Kim EH, Yum MS, Ra YS, Park JB, Ahn JS, Kim GH, et al. Importance of *RNF213* polymorphism on clinical features and long-term outcome in Moyamoya disease. J Neurosurg. 2016;124:1221–7.
23. Bang OY, Chung JW, Cha J, Lee MJ, Yeon JY, Ki CS, et al. A polymorphism in *RNF213* is a susceptibility gene for intracranial atherosclerosis. PLoS One. 2016;11:e0156607.
24. Wu Z, Jiang H, Zhang L, Xu X, Zhang X, Kang Z, et al. Molecular analysis of *RNF213* gene for Moyamoya disease in the Chinese Han population. PLoS One. 2012;7:e48179.
25. Wang X, Zhang Z, Liu W, Xiong Y, Sun W, Huang X, et al. Impacts and interactions of PDGFRB, MMP-3, TIMP-2, and *RNF213* polymorphisms on the risk of Moyamoya disease in Han Chinese human subjects. Gene. 2013;526:437–42.
26. Shang D, Shi C, Mao C, Qin J, Gao Y, Zhao L, et al. Association between ring finger protein 213 gene polymorphism and ischemic stroke in a Chinese Han population. J Apoplexy Nerv Dis. 2015;32:594–7. In Chinese
27. Huang Y, Cheng D, Zhang J, Xiao Y, Luo M. Association between *RNF213* gene polymorphisms and the genetic susceptibility of adult Moyamoya disease of Zhuang population in Guangxi. J Apoplexy Nerv Dis. 2015;32:918–21. In Chinese
28. Zhang Q, Liu Y, Zhang D, Wang R, Zhang Y, Wang S, et al. *RNF213* as the major susceptibility gene for Chinese patients with Moyamoya disease and its clinical relevance. J Neurosurg. 2016 Apr;29:1–8.
29. Lee MJ, Chen YF, Fan PC, Wang KC, Wang K, Wang J, et al. Mutation genotypes of *RNF213* gene from Moyamoya patients in Taiwan. J Neurol Sci. 2015;353(1–2):161–5.
30. Liu W, Senevirathna ST, Hitomi T, Kobayashi H, Roder C, Herzig R, et al. Genome-wide association study identifies no major founder variant in Caucasian Moyamoya disease. J Genet. 2013;92(3):605–9.
31. Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, et al. *RNF213* rare variants in an ethnically diverse population with Moyamoya disease. Stroke. 2014;45:3200–7.
32. Shoemaker LD, Clark MJ, Patwardhan A, Chandratillake G, Garcia S, Chen R, et al. Disease variant landscape of a large multiethnic population of Moyamoya patients by exome sequencing. G3 (Bethesda). 2015;6:41–9.

Part III
Biochemistry, Function and Molecular Pathology

Chapter 4
Molecular Biology of Mysterin/RNF213

Daisuke Morito and Kazuhiro Nagata

Abstract Mysterin, also called RNF213, is a large intracellular protein (591 kDa) that contains two tandem AAA+ ATPase modules and a RING finger ubiquitin ligase motif. Although its physiological role remains unclear, mysterin is considered to be the most prominent susceptibility factor for moyamoya disease, an idiopathic cerebrovascular disorder in humans. The C-terminal R4810K mutation of mysterin significantly increases the risk of moyamoya disease, but its molecular effect on the mysterin protein remains largely unclear. Multiple studies have explored the physiological and pathological roles of mysterin at the molecular, cellular, and tissue/individual levels since its identification and molecular cloning, and the results obtained to date suggest that mysterin is involved in angiogenesis and/or endothelial cell behavior; however, no unified and precise understanding has yet been established. In this chapter, we provide an overview of mysterin's genomic composition, enzymatic activities, structure, and mutant phenotypes in vitro and in vivo and discuss its potential physiological and pathological roles from the standpoint of molecular biology.

Keywords Mysterin • RNF213 • Moyamoya disease • AAA+ protein • Ubiquitin ligase

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4.1 Composition of the *mysterin* Gene

Extensive genetic analyses of moyamoya disease ultimately succeeded in identifying a rare missense single-nucleotide polymorphism (SNP), R4810K, on human chromosome 17 [1–4]. The SNP significantly elevates the risk of moyamoya disease and is considered to be the most prominent risk factor for the disorder (the symptomology and epidemiology of moyamoya disease are described in the preceding chapters and the literature [5]). A very large gene that has come to be known as *RNF213* (hereafter referred to as *mysterin*, moyamoya steno-occlusive disease-associated AAA+ and RING finger protein [6]) was shown to harbor the R4810K mutation, but the composition of the gene had not been fully elucidated even after completion of the human genome project. Instead, it was improperly annotated as two shorter genes, *ALO17* and *RNF213* (not identical to the currently recognized full-length *mysterin* /*RNF213* gene), until its first complete molecular cloning by our group (Fig. 4.1) [3].

ALO17 was identified in 2002 as a fusion partner of anaplastic lymphoma kinase (*ALK*) in anaplastic large-cell lymphoma [7]. Its partial cloning and prediction based on human expressed sequence tag (EST) clones suggested that *ALO17* encodes a protein consisting of 1550 or 1599 amino acids (two alternative splicing isoforms), of which the N-terminal region overlaps with the mysterin protein. However, a subsequent Northern blot analysis using a probe targeting the 5′ region of mysterin did not detect any significant expression of a shorter isoform corresponding to *ALO17* [3], suggesting that the previously identified *ALO17* locus does not constitute a bona fide gene but instead arose from imprecise EST-based prediction. The alternative exon splicing in *ALO17* was predicted to clip 49 amino acids off the ALO17 polypeptide and was reproducibly detected as skipping of exon 4 of *mysterin* [3]. This alternative splicing results in generation of the predominant short and the less abundant long isoforms of full-length mysterin, with lengths of 5207 and 5256 amino acids, respectively. The R4810K mutation in the major mysterin

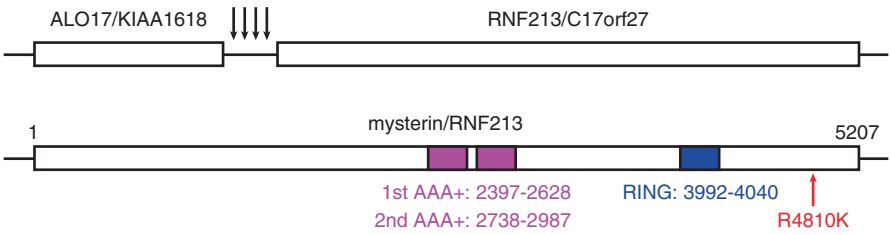

Fig. 4.1 Composition of the *mysterin* gene. *ALO17* and *RNF213* were previously recognized as independent genes (*upper figure*). We identified four unidentified exons (*arrows*) between the two genes by RT-PCR and sequencing and found that these erroneously annotated units constitute a single gene (*lower figure*). The encoded protein, which we named mysterin (moyamoya steno-occlusive disease-associated AAA+ and RING finger protein), harbors two tandem AAA+ modules and a RING finger domain. The C-terminal R4810K mutation significantly increases the risk of moyamoya disease

isoform corresponds to the R4859K mutation in the minor isoform [3, 4]. The role of the mysterin N-terminal region fused with ALK in anaplastic large-cell lymphoma remains largely unclear.

The gene previously annotated as immediately downstream of *ALO17* was *RNF213*, which was predicted to encode a polypeptide with a length of 3602 amino acids, although this polypeptide was not identical to full-length mysterin (Fig. 4.1). The previously recognized *RNF213* is a false gene that was annotated without sufficient experimental assessment. Although neither molecular evaluation nor cloning of the initially identified *RNF213* gene had been reported, this genetic unit was classified as a sensitive genetic marker for phylogenic studies of acanthomorph teleosts (i.e., teleost fish with spiny rays, including many well-known species such as flounder) [8]. The true status of the locus was ultimately elucidated through sequencing of reverse transcription polymerase chain reaction (RT-PCR) products during the molecular cloning of *mysterin* [3]. Our current understanding is that the previous *ALO17* and previous *RNF213* genes in fact constitute a single large gene, *mysterin*, consisting of 68 or 69 exons.

4.2 Molecular Properties of Mysterin Protein

The *mysterin* gene encodes an intracellular protein with multiple unique properties. One immediately obvious feature is its enormous size, 5207/5256 amino acids, making it the 21st longest of 20,191 reliable gene products encoded by the human genome (UniProt: http://www.uniprot.org/). Furthermore, mysterin protein forms a putative hexameric oligomer with a predicted molecular mass of 3.5 MDa [6]. This size is comparable to that of the ribosome, the largest class of macromolecular complex in all living organisms. Mysterin also has a unique combination of enzymatic activities, containing two mechanical-type ATPase modules and a single ubiquitin ligase domain (Fig. 4.1). To date, no other enzyme has been shown to harbor these two types of domains. How this enormous protein coordinates energy-dependent mechanical activity and protein modification/regulation activity, as well as how it contributes to physiological/pathological processes in the cell, represent intriguing questions in molecular and cellular biology.

4.2.1 AAA+ ATPase Activity of Mysterin Protein

Early studies, and even some recent work, reported that mysterin contains a single ATPase domain consisting of approximately a hundred amino acids [3, 4]. This domain harbors a nucleoside triphosphate (NTP)-binding Walker A motif and a magnesium ion-binding Walker B motif, which together constitute a P-loop NTPase domain and cooperatively hydrolyze NTP. Although BLAST analysis detected a single compact NTPase domain at the site, recent structural prediction detected

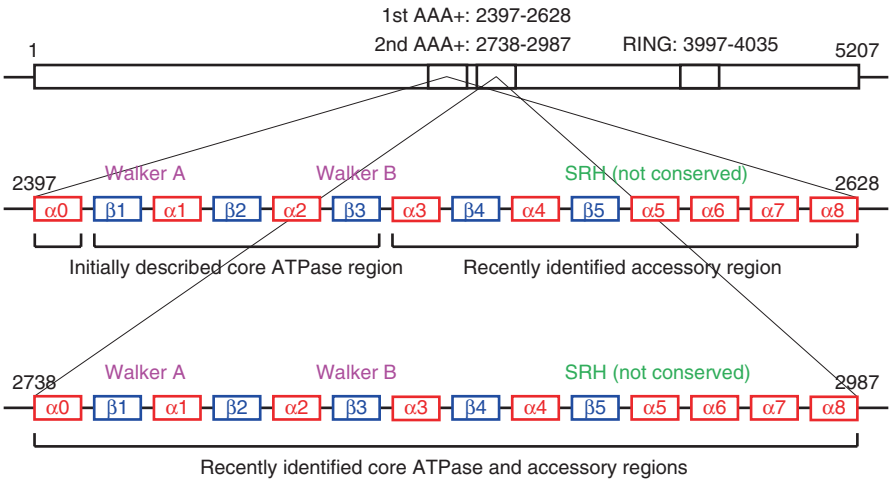

Fig. 4.2 The two tandem AAA+ modules of mysterin. Each AAA+ module consists of nine α-helical and five β-strand regions, whose order and number are similar to those in typical AAA+/AAA modules. The two modules of mysterin preserve essential sequences in the Walker A and B motifs, but not the second region of homology (SRH) motifs that define classical AAA modules. Thus, mysterin is a derivative, rather than canonical, AAA protein. BLAST analysis detects only the former region of the first AAA+ module. The two AAA+ modules are connected by a characteristic helical middle region around a hundred amino acids in length

additional accessory structures around the NTPase domain that are not detectable by BLAST (Fig. 4.2) [6]. Together, the NTPase core motifs and accessory structures constitute an AAA+ (ATPases associated with diverse cellular activities) module, a subclass of the P-loop NTPase domain, over 200 amino acids long [9]. Surprisingly, structural prediction of the entire mysterin polypeptide identified another AAA+ module, including a set of Walker motifs, immediately C-terminal to the first AAA+ module; BLAST analysis had also failed to detect the second module (Fig. 4.2). In vitro enzymatic assay confirmed that both modules exhibit significant ATP hydrolysis activity [6]. Thus, mysterin harbors tandem AAA+ modules, and the failure of BLAST analysis to detect these features has caused mild confusion in the field (i.e., some of the literature states that mysterin contains a single compact ATPase module). It should be noted that mysterin lacks a second region of homology (SRH) motif, a consensus element of canonical AAA proteins, and is thus classified as a derivative AAA+ protein rather than classical AAA protein (Fig. 4.2) [9]. This issue has also been the subject of several errors in the literature.

Two common characteristics of the AAA+ proteins, including the classical AAA proteins, are formation of toroidal oligomers (usually hexamers) and mediation of various biophysical/mechanical processes in the cell via dynamic changes in overall structure coupled to ATP hydrolysis [10]. For example, dynein forms an intramolecular toroidal structure with its six intrinsic AAA+ modules and moves directionally on microtubules using energy from ATP hydrolysis (Fig. 4.3a). ClpB forms a hexameric toroidal oligomer through its tandem AAA+ modules and threads

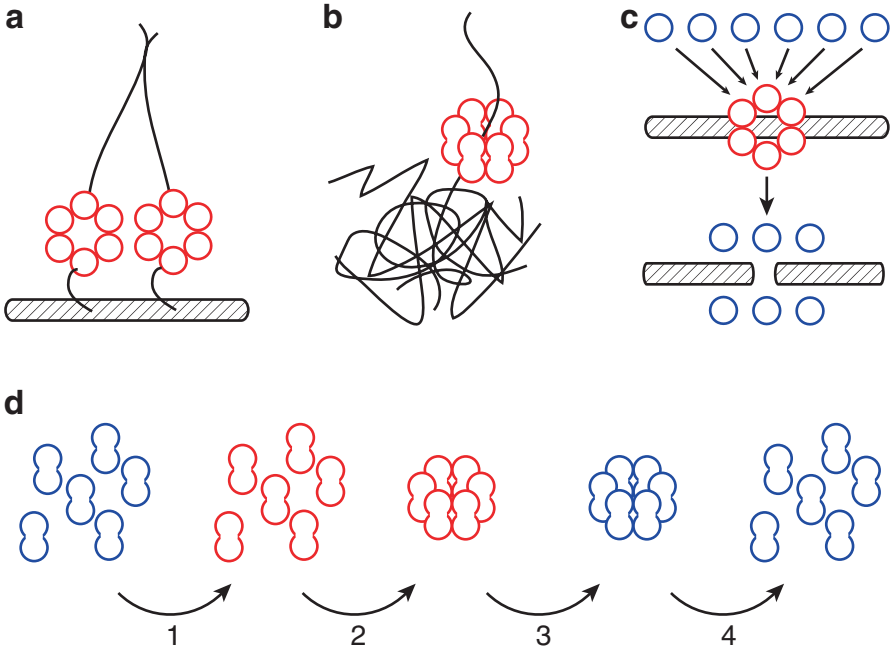

Fig. 4.3 Diverse modalities of AAA+ activities. (**a**) Dynein, which harbors six AAA+ modules in a single heavy chain molecule, forms an intramolecular ring structure and generates motive force for directed motion on microtubule filaments (*striped*). Two dynein heavy chains form a 1.2 MDa complex with other chains. (**b**) Bacterial ClpB and yeast Hsp104 harbor two tandem AAA+ modules connected by a long helical middle region as in mysterin. These proteins form hexamers and pull polypeptides from protein aggregates in cooperation with other molecular chaperones such as Hsp70, resulting in resolution and detoxification of protein aggregates. This function is often referred to as unfoldase activity. (**c**) Katanin remains in the monomeric or dimeric (not shown) state in the cytosol and forms hexamers on its substrate, microtubule filaments. ATP-bound katanin (*red*) dissects microtubule filaments via ATP hydrolysis, and the resultant ADP-bound katanin returns to the diffuse monomeric or dimeric state (*blue*). (**d**) Hypothetical dynamics of mysterin. Mysterin routinely remains in the ADP-bound monomeric state (*blue*) and is converted to the ATP-binding form (*red*) (1). ATP binding by the first AAA+ module triggers oligomer formation (2). The mysterin oligomer hydrolyzes ATP and presumably mediates an unidentified biophysical process in the cell (3, ATP to ADP state). ATP hydrolysis on the second AAA+ module triggers disassembly of mysterin oligomer (4)

polypeptides through its center pore, resulting in resolution of entangled polypeptide aggregates (Fig. 4.3b). Katanin assembles on its substrate, microtubule filaments, and physically dissects them (Fig. 4.3c). Vps4 also assembles on the substrate ESCRT-III complex and drives membrane neck constriction by dissociating the ESCRT-III complex. Thus, AAA+ proteins mediate a wide variety of mechanical processes in the cell, whereas the process mediated by mysterin has not yet been identified. Intriguingly, the two AAA+ modules of mysterin are connected by a helical middle region of about a hundred amino acids resembling an analogous region

in ClpB (Fig. 4.2) that regulates the protein's overall activity [11]. The middle region of mysterin may also play a role in regulating its activity.

Most known AAA+/AAA proteins form stable toroidal oligomers (as noted above, usually hexamers) in the presence of physiological concentrations of ATP and magnesium ions, but the situation is somewhat different for mysterin [6]. Electron microscopic study demonstrated that purified full-length mysterin has the ability to form a toroidal oligomer comparable in size to the ribosome. However, biophysical and biochemical evaluations demonstrated that most mysterin molecules in the cell remain in the monomer state. Although more precise and multifaceted analyses of this issue are warranted, the current biochemical data suggest that mysterin forms oligomers with ATP bound to its first AAA+ module and disassembles with ATP hydrolysis by its second AAA+ module (Fig. 4.3d) [6]. This cyclic oligomer formation and dissociation resembles that of katanin (Fig. 4.3c) and Vps4, which routinely remain in the monomeric state and assemble on their substrates (microtubules or ESCRT-III complex, respectively); they then mediate disassembly of the substrates via ATP hydrolysis and return to the monomeric/dimeric state [10]. Although the role of the AAA+ modules of mysterin remains unclear, recent studies suggest that they are physiologically and pathologically important in vivo, as discussed in the next chapter.

4.2.2 Ubiquitin Ligase Activity of Mysterin Protein

The other prominent domain structure in mysterin is a really interesting new gene (RING) finger domain (Fig. 4.1), through which mysterin exerts its ubiquitin ligase activity [3]. This class of enzyme is involved in the last step of protein ubiquitylation (Fig. 4.4a) [12]. During this process, a small protein (ubiquitin) is initially activated by ubiquitin-activating enzyme (E1) in an energy-dependent manner, transferred to a ubiquitin-conjugating enzyme (E2), and ultimately covalently conjugated to a substrate protein by a ubiquitin ligase (E3) [13]. The target site for ubiquitylation is typically a lysine residue in the substrate protein. Although ubiquitylation is a "one-shot deal" in some cases (monoubiquitylation), multiple cycles of ubiquitylation can occur on a lysine residue of a ubiquitin that is already conjugated to a target protein, resulting in generation of a polyubiquitin chain on the substrate (polyubiquitylation) (Fig. 4.4a). In this situation, one of the seven lysine residues in ubiquitin is selectively ubiquitylated, leading to formation of a polyubiquitin chain with a particular type of chain linkage [14]. The best understood modality of polyubiquitylation is mediated by the 48th lysine of ubiquitin (K48-linked polyubiquitylation), and it is well established that K48-linked polyubiquitin chains serve as a protein degradation signal [13]. The proteasome specifically recognizes K48-linked polyubiquitin chains and degrades ubiquitylated substrates into short polypeptides. An E3 usually exhibits enzymatic activity in cooperation with one or a few specific E2s and generates polyubiquitin chains of one or more types. Such selectivity for E2s and modalities of polyubiquitylation are often associated with substrates or

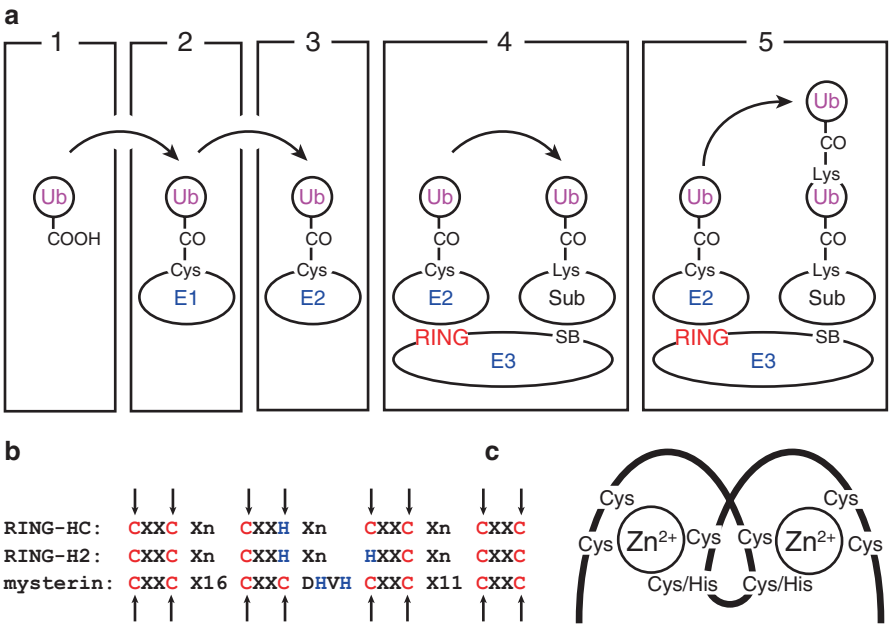

Fig. 4.4 Multistep ubiquitylation reaction and structure of RING finger protein. (**a**) Ubiquitin is a small protein, 76 amino acids in length (1). Its C-terminus is chemically activated by ubiquitin-activating enzyme (E1) using ATP (2). Ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2) (3). A specific type of ubiquitin ligase (E3) harboring a RING finger domain and substrate-binding domain (SB) mediates transfer of ubiquitin from E2 to a lysine residue of the substrate (4). Another cycle of ubiquitin transfer generates a polyubiquitin chain on the substrate (5). (**b**) Eight cysteine (*red*) and histidine (*blue*) residues (other residues: X) are conserved in a single RING finger domain. The RING finger in mysterin includes eight cysteine residues and belongs to the RING-C2 subgroup, although we cannot exclude the possibility that two histidine residues around the center of the RING finger contribute to zinc binding. (**c**) The RING finger domain clasps two ions of zinc to form a "cross-brace" structure

biological conditions, as well as the intrinsic characteristics of the E3 itself. In addition to the major modality of ubiquitylation described above, many exceptional instances with biological relevance have been identified, and these have been extensively described in the protein ubiquitylation literature [14]. Although mysterin exhibits obvious ubiquitin ligase activity, its partner E2 and modality of ubiquitylation, as well as its in vivo substrates, remain largely unknown.

From a structural standpoint, mysterin belongs to a rare subclass of RING finger E3s. The RING finger domain forms a structure analogous to the zinc finger domain [15]. Canonical types of RING finger domains, which consist of dozens of amino acids, are referred to as RING-C3HC4 (or RING-HC) and RING-C3H2C3 (or RING-H2) according to the number and the order of conserved cysteine (C) and histidine (H) residues (Fig. 4.4b) [12]. The domain clasps two atoms of zinc with eight ligands (cysteine and histidine residues) in a "cross-brace" orientation, thus creating the binding surface for E2 (Fig. 4.4c) [15]. However, human mysterin

includes eight cysteines and no histidines at the predicted zinc chelation sites. To date, this orientation, referred to as RING-C4C4 (or RING-C2), has been detected in only a small number of RING family members [16]. The characteristic composition of the mysterin RING finger domain might affect its ubiquitylation modality or biological role, which remain elusive.

4.3 Biological Role of Mysterin

Our knowledge of the physiological and pathological relevance of mysterin still remains limited. The protein's substrates, its contribution to intra- and intercellular processes, and the effects of disease-associated mutations remain largely unclear. Multiple studies have investigated these issues at the molecular, cellular, and tissue/organism levels, and proposed potential roles of mysterin on the basis of their findings, but a precise and unified understanding remains to be established. In this section, we review functional studies of mysterin at multiple levels and discuss the protein's potential physiological and pathological roles.

4.3.1 Potential Physiological Roles

The involvement of the mysterin mutation in human moyamoya cerebrovascular disease led us to examine mysterin's contribution to the physiological structure and/or function of blood vessels. Knockdown and knockout of the *mysterin* gene resulted in abnormal vascular morphology in zebra fish embryos, supporting the hypothesis that moyamoya disease is caused by functional aberration of a gene physiologically involved in blood vessels [3, 17]. Vascular network formation is supported by two fundamental processes, vasculogenesis and angiogenesis [18]. The former includes differentiation of hemangioblasts into vascular endothelial cells and formation of primitive vascular plexus, whereas the latter is initiated by migration and proliferation of vascular endothelial cells from preexisting vascular plexus. Mysterin knockdown/knockout zebra fish embryos retain the ability to develop blood vessels via both processes, although they exhibit somewhat excessive and broadly misguided angiogenesis [3, 17], suggesting that mysterin plays a role in vascular patterning, rather than vascular outgrowth, including differentiation, proliferation, and migration of vascular endothelial cells. Patterning in angiogenesis is determined by the balance of extracellular attractive and repulsive signals, as well as by direct communication between the tip and stalk cells in growing blood vessels [19–21]. Mysterin may play a role in regulatory pathways mediated by cell surface receptors such as VEGF receptor, Plexin, and Notch (also discussed in the next chapter). Further studies are necessary to elucidate the molecular mechanism underlying the vascular phenotypes of mysterin mutants in zebra fish.

Despite the obvious phenotype in zebra fish, the involvement of mysterin in vascular structure and/or function in mammals remains controversial. Mysterin knockout mice exhibit no spontaneous vascular phenotype and few if any vascular anomalies under ischemic conditions [22–25], and mysterin knockdown causes little or no interference with proliferation and tube formation of human endothelial cells in vitro [26–28]. Although the reason for this discrepancy between the zebra fish and mammalian systems remains unclear, mice are often less sensitive to gene targeting than zebra fish, presumably owing to one or more mechanisms that can compensate for loss of the gene function. Further elucidation of the vascular function of mouse/human mysterin is warranted.

Other potential target processes of mysterin have been proposed, including insulin production and pancreatic β-cell survival [22], matrix metalloproteinase production in vascular endothelial cells [24, 28], zebra fish myogenesis [29], WNT and calcium signaling in the endothelial cells that mediate vascular regression [30], and non-mitochondrial oxygen consumption in breast cancer [31]. Here again, however, the underlying mechanisms mostly remain elusive.

Mysterin is conserved across vertebrates and ubiquitously expressed in multiple tissues in human and mouse [3, 4]. Mysterin is distributed throughout the cytosol, and a subset of mysterin molecules is associated with unidentified intracellular structures [3]. Although typical AAA+ ATPases exert their biological activity in the form of toroidal oligomers, most mysterin remains in a monomeric state despite its ability to form such oligomers [6], suggesting that it exhibits its biological activity at a limited number of sites or upon specific stimulation. As discussed in the next chapter, the ATPase and ubiquitin ligase activities of mysterin are both prerequisite for zebra fish organogenesis [29], suggesting that they play indispensable roles in vivo. Future in vitro and in vivo studies should seek to elucidate its target substrate, site(s), and process(es).

4.3.2 Potential Pathogenic Roles

Genetic studies have revealed that the R4810K SNP significantly elevates the risk of moyamoya disease, although the underlying molecular and cellular mechanism remains largely unclear. Because arginine (R) and lysine (K) share similar structural and electrical properties (Fig. 4.5), RK mutations are generally predicted to have limited effects on the biochemical properties of proteins. Our group has failed to detect significant changes in stability, subcellular distribution, ubiquitin ligase activity, and oligomer formation of the R4810K mutant in cells [3, 6], whereas Koizumi and colleagues reported that the mutation negatively affects the ATP hydrolysis activity of the first AAA+ module [32] and impairs cell-cycle progression by interfering with mitotic factors such as Securin and MAD2, resulting in reduced endothelial cell proliferation and angiogenesis in vitro [26, 27]. Notably, some of these results were obtained using patient iPS-derived endothelial cells. Transgenic mice that harbor a mutation corresponding to human R4810K exhibit no spontaneous

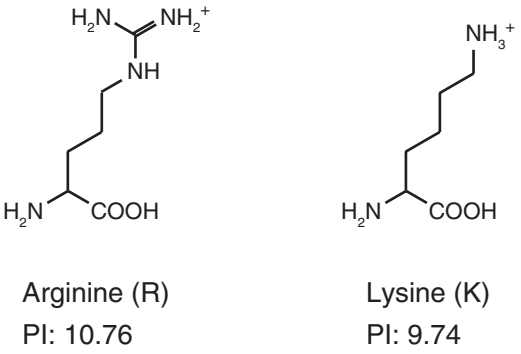

Fig. 4.5 Structures and electrical properties of arginine and lysine. Both amino acid residues have similar isoelectric points (PI), ~10, and are therefore positively charged at neutral pH

vascular anomalies and no/modest inhibition of angiogenesis under ischemic conditions [32, 33]. Thus, involvement of wild-type and mutant mysterin in mammalian angiogenesis still remains controversial, but the R4810K mutation may modulate its intrinsic angiogenic activity or confer a novel activity (i.e., a negative effect on angiogenesis) on mysterin in mammals. Future studies are warranted to elucidate the molecular effect of the R4810K mutation and the roles of wild-type and mutant mysterin in angiogenesis in vitro and in vivo.

It was reported that R4810K homozygosity is positively associated with the disease severity, suggesting that higher expression of mutant mysterin causes more severe vascular anomaly [34]. Recent studies showed that mysterin is strongly induced in endothelial cells by stimulation of interferons β and γ [28, 32]. Upregulation of mutant mysterin upon the inflammatory stimulation may perturb endothermal cell proliferation and/or survival and contribute to more severe endothelial dysfunction in moyamoya disease. Previous report identified low penetrance of inherited moyamoya disease, which also suggests involvement of additional genetic and/or environmental factor(s) [35]. Inflammatory response is a candidate factor that possibly contributes to the onset and progression of moyamoya disease.

4.3.3 Perspectives

After identification of the R4810K mutation by Koizumi and colleagues, our group successfully performed the first complete molecular cloning of the full-length *mysterin* gene [3]. The gene product has a number of intriguing properties such as its enormous size, unique combination of enzymatic activities, and involvement in a mysterious human disease. These features inspired us and other researchers to further explore its physiological and pathological relevance at the molecular, cellular, and organismal levels. Our knowledge of mysterin is still limited, but a top-down approach using animal models and bottom-up approach from the molecular and cellular levels should complement each other, allowing us to fully comprehend the physiological and pathological roles of mysterin in the future.

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References

1. Yamauchi T, Tada M, Houkin K, Tanaka T, Nakamura Y, Kuroda S, et al. Linkage of familial moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. Stroke. 2000;31(4):930–5.
2. Mineharu Y, Liu W, Inoue K, Matsuura N, Inoue S, Takenaka K, et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70(24 Pt 2):2357–63. doi:10.1212/01.wnl.0000291012.49986.f9.
3. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542.
4. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40. doi:10.1038/jhg.2010.132.
5. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7(11):1056–66. doi:10.1016/S1474-4422(08)70240-0.
6. Morito D, Nishikawa K, Hoseki J, Kitamura A, Kotani Y, Kiso K, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. Sci Rep. 2014;4:4442. doi:10.1038/srep04442.
7. Moritake H, Shimonodan H, Marutsuka K, Kamimura S, Kojima H, Nunoi H. C-MYC rearrangement may induce an aggressive phenotype in anaplastic lymphoma kinase positive anaplastic large cell lymphoma: identification of a novel fusion gene ALO17/C-MYC. Am J Hematol. 2002;86(1):75–8. doi:10.1002/ajh.21887.
8. Li B, Dettai A, Cruaud C, Couloux A, Desoutter-Meniger M, Lecointre G. RNF213, a new nuclear marker for acanthomorph phylogeny. Mol Phylogenet Evol. 2009;50(2):345–63. doi:10.1016/j.ympev.2008.11.013.
9. Hanson PI, Whiteheart SW. AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol. 2005;6(7):519–29. doi:10.1038/nrm1684.
10. Ogura T, Wilkinson AJ. AAA+ superfamily ATPases: common structure–diverse function. Genes Cells. 2001;6(7):575–97.
11. Oguchi Y, Kummer E, Seyffer F, Berynskyy M, Anstett B, Zahn R, et al. A tightly regulated molecular toggle controls AAA+ disaggregase. Nat Struct Mol Biol. 2012;19(12):1338–46. doi:10.1038/nsmb.2441.
12. Joazeiro CA, Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. Cell. 2000;102(5):549–52.
13. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem. 1998;67:425–79. doi:10.1146/annurev.biochem.67.1.425.
14. Akutsu M, Dikic I, Bremm A. Ubiquitin chain diversity at a glance. J Cell Sci. 2016;129(5):875–80. doi:10.1242/jcs.183954.
15. Borden KL, Freemont PS. The RING finger domain: a recent example of a sequence-structure family. Curr Opin Struct Biol. 1996;6(3):395–401.
16. van de Weijer ML, Bassik MC, Luteijn RD, Voorburg CM, Lohuis MA, Kremmer E, et al. A high-coverage shRNA screen identifies TMEM129 as an E3 ligase involved in ER-associated protein degradation. Nat Commun. 2014;5:3832. doi:10.1038/ncomms4832.
17. Wen J, Sun X, Chen H, Liu H, Lai R, Li J, et al. Mutation of rnf213a by TALEN causes abnormal angiogenesis and circulation defects in zebrafish. Brain Res. 1644;2016:70–8. doi:10.1016/j.brainres.2016.04.051.

18. Jain RK. Molecular regulation of vessel maturation. Nat Med. 2003;9(6):685–93. doi:10.1038/nm0603-685.
19. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol. 2003;161(6):1163–77. doi:10.1083/jcb.200302047.
20. Torres-Vazquez J, Gitler AD, Fraser SD, Berk JD, Van NP, Fishman MC, et al. Semaphorin-plexin signaling guides patterning of the developing vasculature. Dev Cell. 2004;7(1):117–23. doi:10.1016/j.devcel.2004.06.008.
21. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature. 2007;445(7129):776–80. doi:10.1038/nature05571.
22. Kobayashi H, Yamazaki S, Takashima S, Liu W, Okuda H, Yan J, et al. Ablation of Rnf213 retards progression of diabetes in the Akita mouse. Biochem Biophys Res Commun. 2013;432(3):519–25. doi:10.1016/j.bbrc.2013.02.015.
23. Sonobe S, Fujimura M, Niizuma K, Nishijima Y, Ito A, Shimizu H, 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. doi:10.1016/j.brainres.2014.01.011.
24. Sonobe S, Fujimura M, Niizuma K, Fujimura T, Furudate S, Nishijima Y, et al. Increased vascular MMP-9 in mice lacking RNF213: moyamoya disease susceptibility gene. Neuroreport. 2014;25(18):1442–6. doi:10.1097/WNR.0000000000000289.
25. Ito A, Fujimura M, Niizuma K, Kanoke A, Sakata H, Morita-Fujimura Y, et al. Enhanced post-ischemic angiogenesis in mice lacking RNF213; a susceptibility gene for moyamoya disease. Brain Res. 2015;1594:310–20. doi:10.1016/j.brainres.2014.11.014.
26. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, 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(1):13–9. doi:10.1016/j.bbrc.2013.07.004.
27. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, et al. The moyamoya disease susceptibility variant RNF213 R4810K (rs112735431) induces genomic instability by mitotic abnormality. Biochem Biophys Res Commun. 2013;439(4):419–26. doi:10.1016/j.bbrc.2013.08.067.
28. Ohkubo K, Sakai Y, Inoue H, Akamine S, Ishizaki Y, Matsushita Y, et al. Moyamoya disease susceptibility gene RNF213 links inflammatory and angiogenic signals in endothelial cells. Sci Rep. 2015;5:13191. doi:10.1038/srep13191.
29. Kotani Y, Morito D, Yamazaki S, Ogino K, Kawakami K, Takashima S, et al. Neuromuscular regulation in zebrafish by a large AAA+ ATPase/ubiquitin ligase, mysterin/RNF213. Sci Rep. 2015;5:16161. doi:10.1038/srep16161.
30. Scholz B, Korn C, Wojtarowicz J, Mogler C, Augustin I, Boutros M, et al. Endothelial RSPO3 controls vascular stability and pruning through Non-canonical WNT/Ca(2+)/NFAT signaling. Dev Cell. 2016;36(1):79–93. doi:10.1016/j.devcel.2015.12.015.
31. Banh RS, Iorio C, Marcotte R, Xu Y, Cojocari D, Rahman AA, et al. PTP1B controls non-mitochondrial oxygen consumption by regulating RNF213 to promote tumour survival during hypoxia. Nat Cell Biol. 2016;18(7):803–13. doi:10.1038/ncb3376.
32. Kobayashi H, Matsuda Y, Hitomi T, Okuda H, Shioi H, Matsuda T, et al. Biochemical and functional characterization of RNF213 (Mysterin) R4810K, a susceptibility mutation of moyamoya disease, in angiogenesis in vitro and in vivo. J Am Heart Assoc. 2015;4(7) doi:10.1161/JAHA.115.002146.
33. Kanoke A, Fujimura M, Niizuma K, Ito A, Sakata H, Sato-Maeda M, 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. doi:10.1016/j.brainres.2015.07.039.

34. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78(11):803–10. doi:10.1212/WNL.0b013e318249f71f.
35. Mineharu Y, Takenaka K, Yamakawa H, Inoue K, Ikeda H, Kikuta KI, et al. Inheritance pattern of familial moyamoya disease: autosomal dominant mode and genomic imprinting. J Neurol Neurosurg Psychiatry. 2006;77(9):1025–9. doi:10.1136/jnnp.2006.096040.

Chapter 5
Physiological Role of Mysterin/RNF213 in Zebrafish

Daisuke Morito and Kazuhiro Nagata

Abstract Mysterin, also known as RNF213, is a very large intracellular protein of 591 kDa with unknown function. Long-standing genetic analyses ultimately revealed that its C-terminal mutation (R4810K) is the key genetic factor for moyamoya disease, an inheritable idiopathic cerebrovascular disorder with arterial stenosis and occlusion at a limited site in the brain. Although the pathologic mechanism has remained largely unclear, a leading hypothesis is that mysterin plays a role in the blood vessel and that the R4810K mutation interferes with the vascular function of mysterin, resulting in the development of moyamoya disease. The first evidence that mysterin participates in normal vascular structure and/or function was obtained from zebrafish, a powerful model vertebrate. In this chapter, we overview the vascular and other phenotypes of *mysterin* knockdown/knockout zebrafish and discuss its possible roles in vivo.

Keywords Mysterin • RNF213 • Zebrafish • Moyamoya disease • AAA+ protein • Ubiquitin ligase

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5.1 Introduction

RNF213 (hereafter referred to as mysterin, the name that we have proposed [1]) is a very large intracellular protein with unknown function. It exhibits mechanical ATPase and protein ubiquitylation activities through two intrinsic AAA+ ATPase modules and a RING finger ubiquitin ligase motif, respectively [1, 2]. The gene encoding mysterin was originally identified and cloned after extensive genetic analyses of moyamoya disease, a human idiopathic cerebrovascular disorder that runs in several families [2–5]. As discussed in other chapters of this book and the literature, moyamoya disease is characterized by the progressive stenosis and occlusion of a specific region of the cerebral artery, resulting in the relapsing cerebral infarction, ischemic stroke, and the formation of fragile collateral capillary vessels that cause severe intracranial hemorrhage [6]. Although genetic analyses have revealed that a mutation in the C-terminal region of mysterin (R4810K) significantly increases the risk of moyamoya disease by 100- to 200-fold, its pathophysiological mechanism has remained largely unclear. A leading hypothesis is that mysterin facilitates physiological vascular structure/function in vivo and that the R4810K mutation interferes with the vascular function of mysterin, resulting in the vascular anomalies in moyamoya disease. The first experimental evidence of the functional involvement of mysterin in blood vessels was obtained from the zebrafish, *Danio rerio* [2].

The zebrafish is a small tropical freshwater fish (the adult is ~4–5 cm in length) that is closely related to the carp. Although many genetic studies involve unicellular organisms or invertebrates (some uses plants), several employ the zebrafish, a vertebrate model that is easy to work with, because of its short generation time (~3–4 months), enormous reproductive ability (laying hundreds of eggs per week), and usefulness in a variety of genetic techniques (e.g., mutagenesis, morpholino-mediated transient gene knockdown, transgenesis, gene targeting, genome editing, and transposon technique) [7]. The sequencing of its entire genome, which was completed a few years ago, is another advantage [8]. There are further benefits of using the zebrafish as a research model; the zebrafish has many unique properties such as organs that are similar to those of the human, rapid and external development, and the optical transparency of embryo and mutant adult (i.e., the casper mutant, named after Casper the Friendly Ghost [9]). In particular, the entire body transparency enables straightforward histological analysis of any organs including the blood vessels. The transgenic zebrafish expressing green fluorescent protein (GFP) under the control of the vascular endothelial cell-specific promoter allows researchers to clearly visualize the vascular system even within deep tissues without fixation, sectioning, staining, or autopsy [10]. This property allows researchers to easily and extensively explore vascular anomalies in mutants, which, if using the mouse, would need much more effort.

5.2 Preservation and Duplication of the *mysterin* Gene in Zebrafish

Despite the usefulness of the zebrafish, a potential disadvantage is the extra duplication of the entire genome that is unique to teleost fish. The "two rounds (2R) hypothesis," which is now widely accepted, states that the vertebrate genome underwent two rounds of genome duplication at a very early stage of vertebrate evolution (i.e., before the divergence of jawless and jawed vertebrates) [11]. However, teleost fish underwent an additional round of genome duplication, resulting in the creation of a couple of paralogous genes corresponding to a single human gene [12]. The zebrafish retains two *mysterin* genes, *alpha* and *beta*, that are found on different chromosomes, although a single *mysterin* gene is conserved across vertebrates other than teleost fish [2]. The consensus sequences of both sets of Walker NTPase motifs and the single RING finger domain are preserved in zebrafish *mysterin alpha* and *beta* genes (Fig. 5.1), suggesting that the zebrafish mysterin paralogues exhibit enzymatic activities similarly with their human orthologue [13]. Although their tissue distribution patterns and functional divergence/redundancy remain unknown, the alpha isoform is predominantly expressed in the zebrafish embryo, where it plays a significant role in the early formation of blood vessels and the motor system [2, 13].

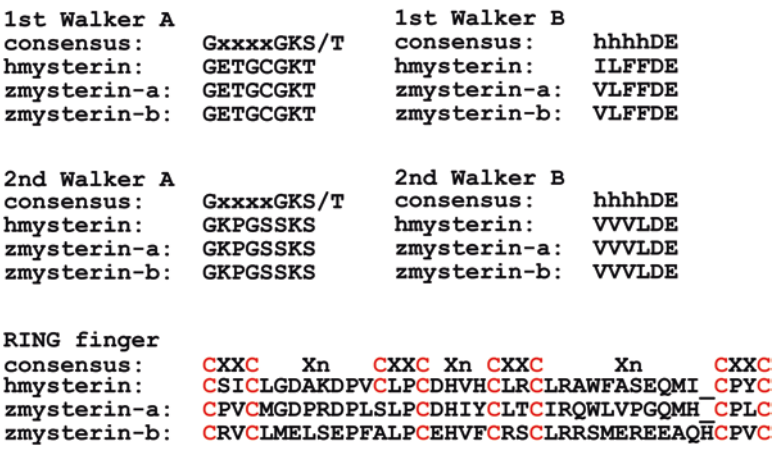

Fig. 5.1 Conserved motifs in human and zebrafish mysterin. ATP-binding Walker A motifs and magnesium ion-binding B motifs in two AAA+ modules are highly conserved in human and zebrafish mysterin. Seven out of eight cysteine residues in the RING finger domain are conserved in zebrafish mysterin alpha and beta (zmysterin-a and zmysterin-b), whereas the third position is replaced with a serine or alanine residue in mysterin alpha and beta, respectively. How these replacements affect the ubiquitin ligase activity of mysterin remains elusive. *x* and *h* indicate any amino acid and any hydrophobic amino acid, respectively

5.3 Mysterin in Zebrafish Angiogenesis

The formation of the vascular system involves two processes, vasculogenesis and angiogenesis, which are common to all vertebrates [14]. Vasculogenesis is the preceding process in which hemangioblasts differentiate into vascular endothelial cells and form the primitive vascular plexus. Angiogenesis, on the other hand, involves the proliferation of vascular endothelial cells and their migration from the preexisting vascular plexus to form sprouting vessels. Although an atlas of the vascular network in the zebrafish, as well as its formation process in ovo, has been precisely described, the underlying molecular mechanisms remain largely unclear [15, 16]. A simple circulatory loop is firstly formed at 1 day postfertilization (dpf), which consists of the dorsal aorta (DA), the cardinal vein, and the primitive cranial vasculature, and mediates early embryonic circulation. Arteriovenous (AV) identity with unique genetic and functional properties is already acquired at this stage of development. Thereafter, the expansion of the vascular network occurs by angiogenesis.

Morpholino antisense oligonucleotides (MOs) were designed to target the exon–intron boundaries in *mysterin alpha* and *beta*, which effectively prevented the proper splicing of each transcription products in vivo and interrupted translation by the introduction of frameshift [2]. The MOs were injected into transgenic zebrafish, whose endothelial cells expressed enhanced GFP (EGFP) under the control of the *fli1* promoter, at the 1- to 8-cell stage. Both *alpha* and *beta* morphants (animals treated with MOs) showed no apparent anomaly in vasculogenesis and AV specification, whereas the *alpha* but not the *beta* morphants showed significant anomalies in angiogenic formation of the intersegmental vessels (ISVs) and the cranial vessels including the inner optic circle (IOC) [2]. ISVs initially emerge from the preexisting DA via angiogenesis and faithfully track somite boundaries (Fig. 5.2a) [15]. The *mysterin alpha* morphants showed normal angiogenic activity, whereas excessive sprouting was observed in several ISVs of the morphants (Fig. 5.2b). Moreover, the

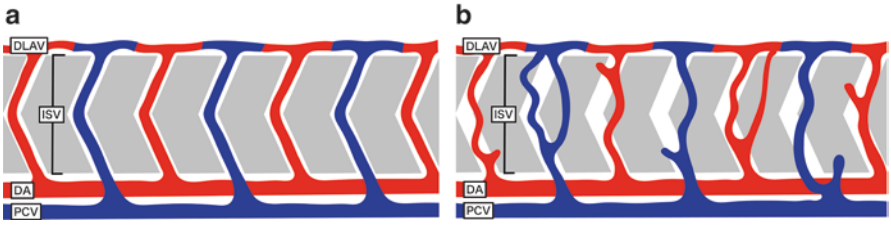

Fig. 5.2 Rough sketch of the intersegmental vessels in the zebrafish. (**a**) The intersegmental vessels (ISVs) initially emerge from the dorsal aorta (DA), grow dorsally along the vertical somite boundaries, and connect to form the dorsal longitudinal anastomotic vessel (DLAV). The connection with the DA is replaced after 1.5 dpf with that of the posterior cardinal vein (PCV). Arteriovenous identities are acquired in response to the directed blood flow (the artery and vein are shown in *red* and *blue*, respectively). (**b**) The ISVs develop by straying from the somite boundaries in the *mysterin* morphants. The morphants also show excessive sprouting (Illustrated by Ayano Kasai)

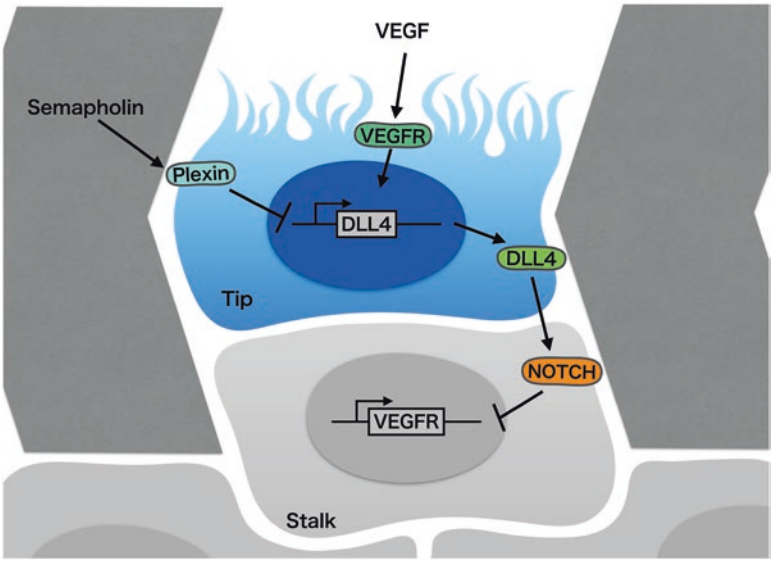

Fig. 5.3 Angiogenic regulatory pathways in the trunk of the zebrafish. The tip cell migrates dorsally after VEGF-induced filopodial formation, leading to angiogenesis dorsally. The tip cell maintains the identity of the stalk cell via Notch–Delta (DLL4: Delta-like 4) signaling not to respond to VEGF. Semaphorin and plexin signaling contributes to vascular patterning via suppression of the DLL4 (Illustrated by Ayano Kasai)

mysterin alpha morphants showed severe straying of ISVs from the somite boundaries, although somite boundaries per se formed normally, and the initiation sites for the vessel sprouting were also normal (Fig. 5.2b). Excessive vascular sprouting was observed also in the nasal ciliary artery (NCA) of the *alpha* morphants, resulting in severe malformation of the IOC. Notably, the malformation of the IOC is a unique phenotype previously not reported in the ZFIN database (https://zfin.org/) [2]. Thus, the *mysterin* morphants showed over-sprouting and mispatterning of ISVs in the trunk region and over-sprouting in IOC. Although questions were recently raised about the reliability of the morpholino technique [17], these phenotypes were recently successfully reproduced in *mysterin alpha* knockout zebrafishes [18].

Our knowledge of mysterin function in angiogenesis in zebrafish is limited, but mysterin function should fall into either of two categories: (1) direct participation in angiogenic pathways that are mediated by canonical signaling factors such as VEGF, Notch, and plexin and (2) indirect participation in angiogenesis via nonclassical mechanisms such as RNA processing and lipid metabolism. As depicted in Fig. 5.3, the tip cell, a subtype of vascular endothelial cell that is distributed at the tip of the developing vasculature, is stimulated by VEGF, leading to angiogenic vascular extension [16]. The tip cell affects the stalk cell through Delta–Notch signaling and prevents conversion of the stalk cell into the second tip cell that may sprout from middle of the developing vessel. The direction of the vessel extension is also affected by the semaphorin/plexin signaling pathway (Fig. 5.3). *Plexin*

mutant zebrafish shows an aberrant ISV patterning similar to that seen in the *mysterin* morphants [19]. Thus, vascular guidance is regulated through the balance between attractive (e.g., VEGF) and potential repulsive (e.g., plexin) signaling that include multiple pathways besides those mentioned in this review [16]. A plausible model is that mysterin alpha is a factor that mildly inhibits basal angiogenic activities such as endothelial cell proliferation and migration under physiological condition, while the other possibility is that mysterin is involved in vascular patterning/guidance rather than in the basal angiogenic activities. Deregulation in vascular elongation, which is physiologically under precise control, may cause both excessive branching and straying from the proper route (e.g., somite boundaries in ISVs).

The second major possibility is that mysterin contributes to vascular formation through nonclassical pathways that have not been suspected to participate in angiogenesis. TDP-43 is a RNA-binding protein that functions in multiple RNA-related processes such as gene expression, alternative splicing, RNA transportation, and stabilization [20]. Loss of TDP-43 in zebrafish surprisingly leads to severe vascular mispatterning in ISVs and the cranial region, although embryogenesis and vasculogenesis proceed normally [21]. The underlying mechanism for the observed phenotype, however, remains unknown. The mutation of a conserved amino acid in microsomal triglyceride transfer protein, which is required for the biosynthesis of ApoB-containing lipoprotein complexes such as the low density lipoprotein (LDL), caused excessive angiogenesis in zebrafish, while the underlying mechanism remains also unknown [22]. Given the involvements of nonclassical factors in the zebrafish angiogenesis, we cannot exclude the possibility that mysterin functions outside of classic endothelial and/or vascular pathways.

5.4 Mysterin in Zebrafish Myogenesis

The *mysterin alpha* morphants showed an interesting phenotype other than vascular anomaly. Most of the *mysterin alpha* morphants did not hatch even at 3 dpf when the control animals had completed hatching [13]. Similar to baby chicks, zebrafish embryos need to physically break out of their eggshells. However, the *mysterin* morphants showed severely reduced motor activity in ovo, which appeared to affect the ability to hatch. We evaluated the motor system including the muscles and motoneurons and found a severely reduced number of myofibrils that led to the formation of thin and fragile muscle fibers (Fig. 5.4a, b) [13]. This anomaly was detected only in the fast muscle and not in the slow muscle (the fast and slow muscles are clearly divided in fish), suggesting that mysterin plays a specific role in the fast muscle. In addition, ectopically expressed wild-type but not enzymatic mutants of mysterin rescued the fast muscle anomaly (Fig. 5.4c), indicating that both enzymatic

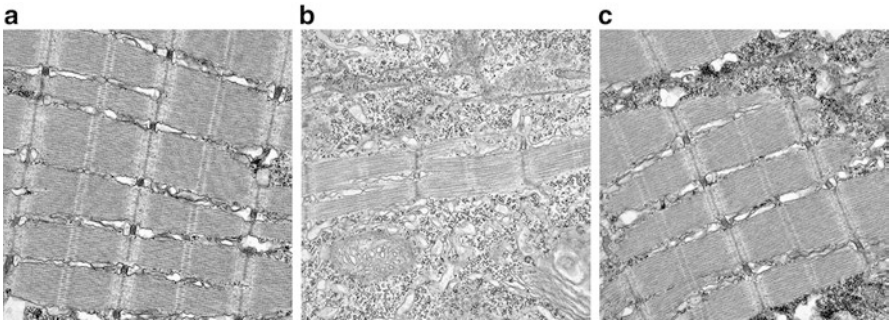

Fig. 5.4 Impaired fast muscle fiber formation and its recovery by ectopic expression of human mysterin. (**a**) Control zebrafish embryos (2 dpf) show thick muscle fibers consisting of multiple myofibrils in an electromicroscopic image of a vertical section of the trunk fast muscle. (**b**) The *mysterin alpha* morphants show thinner muscle fibers consisting of a few myofibrils (a fragile muscle fiber in this image is composed of a single myofibril). (**c**) Ectopic expression of human mysterin in the fast muscle recovers impaired fast muscle formation in the *mysterin* morphants, although enzymatic mutants did not recover the muscle anomaly (data not shown) (These images were obtained simultaneously with those already published [13])

activities of mysterin are involved, while how mysterin participates in myogenesis in zebrafish via the two enzymatic activities remains elusive. The participation of mysterin beta in these processes is also unknown. Notably, the *TDP-43* knockout zebrafish similarly showed thinner muscle fibers, as well as vascular mispatterning, while its phenotypical and functional relationship with *mysterin* remains totally unknown.

5.5 Perspectives

Mysterin knockout mice and knockdown mammalian cells developed no/modest spontaneous vascular/endothelial anomalies [23–29], although zebrafish showed spontaneous and significant vascular phenotypes by *mysterin* knockdown/knockout [2, 13, 18]. We cannot simply apply the vascular and other phenotypes observed in the zebrafishes to mammals, but underlying molecular mechanisms in zebrafish might be translatable to mice and humans. Identifying the molecular mechanisms that underlie the vascular and other anomalies in zebrafish and their reevaluation in mammalian systems might provide key insights into the physiological and pathological roles of mysterin in humans.

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References

1. Morito D, Nishikawa K, Hoseki J, Kitamura A, Kotani Y, Kiso K, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. Sci Rep. 2014;4:4442. doi:10.1038/srep04442.
2. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542.
3. Yamauchi T, Tada M, Houkin K, Tanaka T, Nakamura Y, Kuroda S, et al. Linkage of familial moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. Stroke. 2000;31(4):930–5.
4. Mineharu Y, Liu W, Inoue K, Matsuura N, Inoue S, Takenaka K, et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70(24 Pt 2):2357–63. doi:10.1212/01.wnl.0000291012.49986.f9.
5. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40. doi:10.1038/jhg.2010.132.
6. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7(11):1056–66. doi:10.1016/S1474-4422(08)70240-0.
7. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet. 2007;8(5):353–67. doi:10.1038/nrg2091.
8. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496(7446):498–503. doi:10.1038/nature12111.
9. White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, et al. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell. 2008;2(2):183–9. doi:10.1016/j.stem.2007.11.002.
10. Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. Dev Biol. 2002;248(2):307–18. doi:S0012160602907116 [pii].
11. Taylor JS, Raes J. Duplication and divergence: the evolution of new genes and old ideas. Annu Rev Genet. 2004;38:615–43. doi:10.1146/annurev.genet.38.072902.092831.
12. Jaillon O, Aury JM, Brunet F, Petit JL, Stange-Thomann N, Mauceli E, et al. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature. 2004;431(7011):946–57. doi:10.1038/nature03025.
13. Kotani Y, Morito D, Yamazaki S, Ogino K, Kawakami K, Takashima S, et al. Neuromuscular regulation in zebrafish by a large AAA+ ATPase/ubiquitin ligase, mysterin/RNF213. Sci Rep. 2015;5:16161. doi:10.1038/srep16161.
14. Jain RK. Molecular regulation of vessel maturation. Nat Med. 2003;9(6):685–93. doi:10.1038/nm0603-685.
15. Isogai S, Horiguchi M, Weinstein BM. The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. Dev Biol. 2001;230(2):278–301. doi:10.1006/dbio.2000.9995.
16. Gore AV, Monzo K, Cha YR, Pan W, Weinstein BM. Vascular development in the zebrafish. Cold Spring Harb Perspect Med. 2012;2(5):a006684. doi:10.1101/cshperspect.a006684.
17. Kok FO, Shin M, Ni CW, Gupta A, Grosse AS, van Impel A, et al. Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. Dev Cell. 2015;32(1):97–108. doi:10.1016/j.devcel.2014.11.018.
18. Wen J, Sun X, Chen H, Liu H, Lai R, Li J, et al. Mutation of rnf213a by TALEN causes abnormal angiogenesis and circulation defects in zebrafish. Brain Res. 1644;2016:70–8. doi:10.1016/j.brainres.2016.04.051.
19. Torres-Vazquez J, Gitler AD, Fraser SD, Berk JD, Van NP, Fishman MC, et al. Semaphorin-plexin signaling guides patterning of the developing vasculature. Dev Cell. 2004;7(1):117–23. doi:10.1016/j.devcel.2004.06.008.

20. Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front Biosci. 2008;13:867–78.
21. Schmid B, Hruscha A, Hogl S, Banzhaf-Strathmann J, Strecker K, van der Zee J, et al. Loss of ALS-associated TDP-43 in zebrafish causes muscle degeneration, vascular dysfunction, and reduced motor neuron axon outgrowth. Proc Natl Acad Sci U S A. 2013;110(13):4986–91. doi:10.1073/pnas.1218311110.
22. Avraham-Davidi I, Ely Y, Pham VN, Castranova D, Grunspan M, Malkinson G, et al. ApoB-containing lipoproteins regulate angiogenesis by modulating expression of VEGF receptor 1. Nat Med. 2012;18(6):967–73. doi:10.1038/nm.2759.
23. Kobayashi H, Yamazaki S, Takashima S, Liu W, Okuda H, Yan J, et al. Ablation of Rnf213 retards progression of diabetes in the Akita mouse. Biochem Biophys Res Commun. 2013;432(3):519–25. doi:10.1016/j.bbrc.2013.02.015.
24. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, 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(1):13–9. doi:10.1016/j.bbrc.2013.07.004.
25. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, et al. The moyamoya disease susceptibility variant RNF213 R4810K (rs112735431) induces genomic instability by mitotic abnormality. Biochem Biophys Res Commun. 2013;439(4):419–26. doi:10.1016/j.bbrc.2013.08.067.
26. Sonobe S, Fujimura M, Niizuma K, Nishijima Y, Ito A, Shimizu H, 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. doi:10.1016/j.brainres.2014.01.011.
27. Sonobe S, Fujimura M, Niizuma K, Fujimura T, Furudate S, Nishijima Y, et al. Increased vascular MMP-9 in mice lacking RNF213: moyamoya disease susceptibility gene. Neuroreport. 2014;25(18):1442–6. doi:10.1097/WNR.0000000000000289.
28. Kanoke A, Fujimura M, Niizuma K, Fujimura T, Kakizaki A, Ito A, et al. Temporal profile of magnetic resonance angiography and decreased ratio of regulatory T cells after immunological adjuvant administration to mice lacking RNF213, a susceptibility gene for moyamoya disease. Brain Res. 1642;2016:1–9. doi:10.1016/j.brainres.2016.03.009.
29. Ohkubo K, Sakai Y, Inoue H, Akamine S, Ishizaki Y, Matsushita Y, et al. Moyamoya disease susceptibility gene RNF213 links inflammatory and angiogenic signals in endothelial cells. Sci Rep. 2015;5:13191. doi:10.1038/srep13191.

Chapter 6
Pathological Investigation on *RNF213*: Animal Models Knockout and Transgenic Mice in Diabetes and Signal Transduction

Hatasu Kobayashi

Abstract *RNF213* (*Ring finger protein 213*, *Mysterin*) was identified as a susceptibility gene for moyamoya disease (MMD). The first variant identified in *RNF213* was p.R4810K, which was strongly associated with MMD in East Asian patients. To establish MMD animal models and to investigate the pathological role of *RNF213* in MMD, genetically modified mice have been produced, including the *Rnf213* knockout (KO) mouse, which is based on a loss-of-function model, and *Rnf213* transgenic (Tg) mice, which do not assume a genetic mechanism.

Rnf213 KO mice display thinning of the common carotid artery (CCA) intima and medial layers after CCA ligation, although the cerebrovascular stenotic changes seen in MMD have not been observed. This vascular wall thinness might represent constrictive arterial remodeling, a very early hallmark of MMD. Furthermore, *Rnf213* ablation improves the diabetic phenotype in Akita mice, an endoplasmic reticulum stress-associated diabetic mouse model, by protecting pancreatic β cells, suggesting that *RNF213* is involved in the unfolded protein response.

On the other hand, endothelial cell (EC)-specific mutant *Rnf213* Tg mice, but not EC-specific wild-type *Rnf213* Tg mice, smooth muscle cell-specific mutant *Rnf213* Tg mice, and *Rnf213* KO mice, revealed suppression of responsive cerebral angiogenesis after hypoxia exposure, which is consistent with reduced angiogenesis found in MMD cell models. These results support the hypothesis that RNF213 R4810K overexpression in ECs may induce EC dysfunction, leading to consequent MMD onset.

Conflicting conclusions have been drawn from *Rnf213* KO and Tg mouse studies, and it remains to be determined whether the *Rnf213* KO mouse or Tg mouse is more appropriate as an MMD model. Further studies are needed to establish convincing MMD animal models displaying cerebrovascular stenotic change.

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6.1 Introduction

RNF213 (*Ring finger protein 213*, *Mysterin*) was first reported in 2010 as a susceptibility gene for moyamoya disease (MMD) [1], which was confirmed 1 year later [2, 3]. *RNF213* encodes a relatively large protein that functions both as an AAA+ATPase and an E3 ligase [3, 4]. The founder *RNF213* variant, p.R4810K, was found at high frequency and with strong association in East Asian MMD patients (Japan and Korea, 80%–90%; China, approximately 25%), but was a rare variant in the general East Asian population (Japan and Korea, 2–3%; China, approximately 1%) [3, 5]. Most *RNF213* R4810K carriers are not affected with MMD (MMD incidence was estimated at 1/200–300 carriers), although *RNF213* R4810K carriers develop MMD more frequently than noncarriers (odds ratio is more than 100) [5]. This low penetrance of *RNF213* R4810K could be explained by the involvement of environmental factors in MMD etiology.

To investigate the pathological role of *RNF213* in MMD, genetically modified mice, *Rnf213* knockout (KO) and *Rnf213* transgenic (Tg) mice, have been produced. In this chapter we describe the phenotypes observed in these mice and discuss putative mechanisms involved in these findings.

6.2 *Rnf213* KO Mice

6.2.1 Rnf213 *KO Mice Under Normal Conditions*

RNF213 knockdown zebrafish model using antisense oligos showed abnormal sprouting of vessels in the head region [3]. In addition, fast myofibrils and immature projection of primary motor neurons were reduced, resulting into severe motor deficits [6]. However, under normal conditions, *Rnf213* KO mice did not display any neuromuscular abnormality or cerebrovascular phenotype similar to MMD and appeared to be overtly healthy, even at around 80 weeks of age [7]. Sonobe et al. also showed an absence of any cerebrovascular abnormality in *Rnf213* KO mice by MRA imaging and histopathological analyses [8]. These species differences suggest that *RNF213* is essential for vascular and neuromuscular development in zebrafish, but not in mice. Further studies of these species differences, which might reveal the existence of compensatory pathways in mammals, are required to determine the physiological roles of *RNF213*.

6.2.2 Rnf213 *KO Mice Exposed to Ischemia and Hypoxia*

Rnf213 KO mice have been studied under several stressful conditions, such as artery ligation [8–10] and hypoxia [11]. Following common carotid artery (CCA) ligation, which induces temporary vascular remodeling, thinning of the medial layer and inhibition of intimal hyperplasia in the CCA were observed in *Rnf213* KO mice [8]. The suppression of intimal hyperplasia did not match the stenotic changes observed in MMD; however, the medial layer thinning was consistent with MMD. The authors raised the possibility that thinning of the entire vascular wall, including suppression of intimal hyperplasia, may reflect a very early finding of constrictive remodeling [12], which Kaku et al. recently described as a characteristic of MMD [13]. Ito et al. estimated the effect of ischemia on vascular remodeling in *Rnf213* KO mice [10]. They observed enhanced angiogenesis in the hind limb after permanent femoral artery ligation, but not in the brain after transient middle cerebral artery occlusion. Although conflicting results were obtained between the brain and hind limb, they speculated that *RNF213* influences vascular remodeling in chronic ischemia. Recently, Kobayashi et al. reported that there were no differences in cerebrum angiogenesis between *Rnf213* KO and WT mice exposed to hypoxia [11], indicating an absence of angiogenic change in the brain of *Rnf213* KO mice.

6.2.3 Rnf213 *KO Mice and Diabetes*

To test the effect of *Rnf213* ablation on the angiopathy induced by hyperglycemia, *Rnf213* KO mice were interbred with Akita mice, a diabetic mouse model. Akita mice harbor the *Ins2* C96Y mutation and develop diabetes through endoplasmic reticulum (ER) stress-induced pancreatic β cell apoptosis [14, 15]. Although *Rnf213* KO/Akita mice did not develop cerebrovascular stenotic changes, disruption of *Rnf213* unexpectedly improved glucose tolerance in Akita mice [7]. Plasma and pancreatic insulin levels were higher in *Rnf213* KO/Akita mice than in Akita mice, which was consistent with the observed glucose tolerance improvement. Hyperphagia, a finding associated with diabetes in Akita mice, was reduced in *Rnf213* KO/Akita mice, but levels of plasma leptin, a feeding regulation hormone, were not altered in these mice. These results suggest that hyperphagia was inhibited by elevated plasma insulin concentrations, which stimulate overlapping insulin-leptin signaling pathways in the central nervous system [16]. Interestingly, electron microscopy and immunohistochemistry for insulin and CHOP, a marker for ER stress-associated apoptosis, indicated reduced pancreatic β cell damage in *Rnf213* KO/Akita mice. Collectively, these findings show that disruption of *Rnf213* is likely to improve the diabetic phenotype in Akita mice by protecting β cells from apoptosis.

The *Ins2* C96Y mutation in Akita mice causes a conformational change in the insulin molecule and the resulting ER stress in pancreatic β cells, leading to hyper-

glycemia with reduced β cell mass. ER stress induces an unfolded protein response (UPR), such as degradation of unfolded proteins by ER-associated degradation (ERAD) [17]. The *Ins2*^{C96Y} allele has been reported to act dominantly to enhance degradation of both the C96Y and wild-type proinsulins by the ERAD pathway [18, 19], raising the possibility that ablation of *Rnf213* may impair ERAD and lead to the sparing of wild-type proinsulin. It is thus speculated that *RNF213* may be involved in the ERAD pathway as an E3 ligase in the proteasome.

6.3 *Rnf213* Tg Mice

To obtain an MMD cell model, induced pluripotent stem cells (iPSCs) were established from MMD patients with the *RNF213* R4810K variant. Functional assays using endothelial cells (ECs) differentiated from these iPSCs (iPSECs) revealed inhibition of angiogenesis [20]. Inhibited angiogenesis was also observed in *RNF213* R4810K-overexpressing human umbilical venous endothelial cells (HUVECs) but not in RNF213 WT *RNF213*-overexpressing HUVECs. In contrast, *RNF213* suppression by RNA interference did not reduce angiogenesis in HUVECs, consistent with the absence of vascular phenotypes in *Rnf213* KO mice.

Based on these findings, mice overexpressing *RNF213* R4810K in ECs are considered to be appropriate for an MMD model. Kobayashi et al. produced three types of tissue-specific *Rnf213* Tg mice: vascular EC-specific *Rnf213* R4757K Tg mice (EC-Mut Tg) (R4757K is equivalent to R4810K in human *RNF213*), vascular EC-specific *Rnf213* WT Tg mice (EC-WT Tg), and vascular smooth muscle cell (SMC)-specific *Rnf213* R4757K Tg mice (SMC-Mut Tg) [11]. The authors estimated cerebellar angiogenesis in vivo by capillary staining of the cerebral cortex of mice exposed to hypoxia. Hypoxia experiments were performed in five groups of mice: (1) EC-Mut Tg, (2) EC-WT Tg, (3) SMC-Mut Tg, (4) KO, and (5) WT mice. Hypoxia-induced angiogenesis was inhibited in EC-Mut Tg mice, while angiogenesis was significantly induced by hypoxia in EC-WT Tg, SMC-Mut Tg, KO, and WT mice (Fig. 6.1). In addition, magnetic resonance angiogram (MRA) imaging was performed using these mice under hypoxia. However, no stenotic lesions, moyamoya vessels, or lesions indicative of cerebral infarction were observed in mice of any genotype (Fig. 6.2). These results reproduced the reduced angiogenesis observed in iPSECs from MMD patients [20], suggesting that *RNF213* R4810K is likely to be a gain-of-function rather than a loss-of-function variant. However, a recent cell biological study reports opposite results of an inhibitory effect of *RNF213* knockdown on angiogenesis in HUVECs [21]. Therefore, the genetic mechanism of *RNF213* R4810K remains controversial as to whether RNF213 R4810K is a loss-of-function or gain-of-function mutation. Further studies are needed to resolve these controversies.

Hitomi et al. [20] first established iPSCs from MMD patients with R4810K and non-affected R4810K carriers and demonstrated a deleterious effect of *RNF213* R4810K on angiogenic activity of iPSECs. Microarray analysis showed downregu-

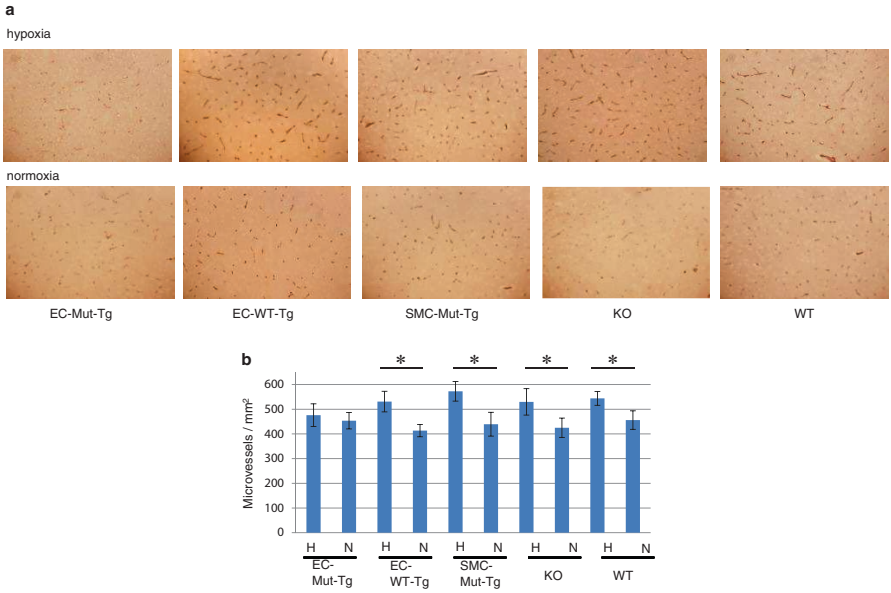

Fig. 6.1 Suppressive effect of in vivo angiogenesis by *Rnf213* mutants in ECs (reproduced from [11]). (**a**) Representative images of microvessels in the cerebral cortex of EC-Mut Tg, EC-WT Tg, SMC-Mut Tg, KO, and WT mice under conditions of normoxia (N) and hypoxia (H). (**b**) Quantification of cerebral microvessels. A column with a *bar* represents the mean ± SD of the number of cerebral microvessels/mm^2 from six mice per group. * $P < 0.05$ according to Mann–Whitney U test compared with normoxia conditions

lation of cell cycle-associated genes in MMD iPSECs [20]; therefore, the authors tested the effect of *RNF213* R4810K on cell cycle function. *RNF213* R4810K overexpression, but not *RNF213* WT overexpression or *RNF213* suppression, inhibited cell proliferation of HUVEC and HeLa cells [22]. Further investigation using HeLa cells demonstrated that *RNF213* R4810K overexpression extended the duration of mitosis and increased the rate of cell division failure, indicating mitotic dysfunction. Mitotic dysfunction was confirmed in iPSECs from MMD patients. In addition, a key regulator of mitosis, mitotic arrest deficiency 2 (MAD2), was found to be co-localized with *RNF213* R4810K during mitosis. *RNF213* R4810K complexes formed with MAD2 were more abundant than those formed with *RNF213* WT. These results suggested that *RNF213* R4810K adversely affected the localization of MAD2 to the kinetochore during mitosis, leading to mitotic abnormality. Furthermore, aneuploidy, a hallmark of genomic instability, occurred more frequently in fibroblasts from MMD patients compared with fibroblasts from control subjects under mitotic arrest induced by an antimitotic agent, nocodazole. Taken together, in MMD patients, *RNF213* R4810K induced mitotic abnormalities and increased the risk of genomic instability, which might be associated with reduced angiogenesis through EC dysfunction and/or apoptosis. It is interesting that cell cycle abnormalities are common to some monogenic diseases showing comorbidity

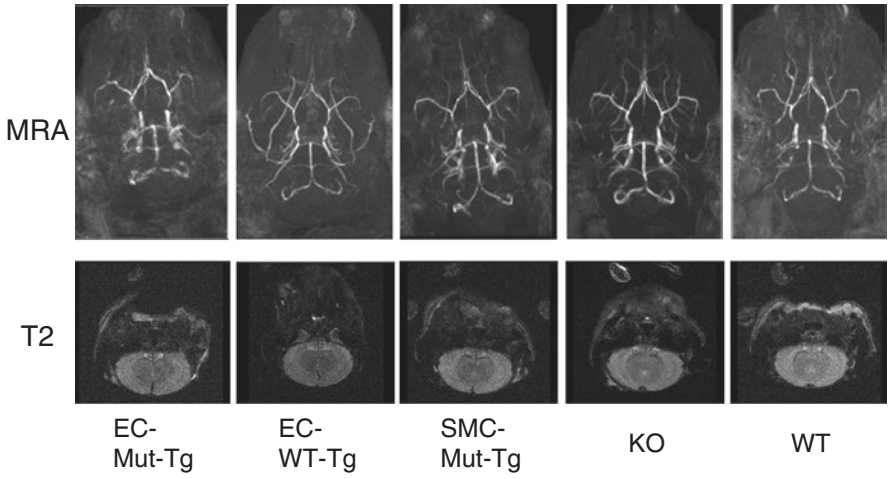

Fig. 6.2 Representative MRI images of the brain of EC-Mut Tg, EC-WT Tg, SMC-Mut Tg, KO, and WT mice with hypoxia (reproduced from [11]). MRA (*upper panel*) represents MRA images. No stenotic lesions or moyamoya vessels were detected in the brain. T2 (*lower panel*) represents T2-weighted images. No infarctions were detected in the brains. Absence of stenotic lesion, moyamoya vessels, and infarction was also confirmed in two other mice of each genotype

with angiopathy like MMD such as Schimke immuno-osseous dysplasia [23], microcephalic osteodysplastic primordial dwarfism type II (MOPDII) [24], and Seckel syndrome [25]. Notably, recent reports demonstrated important roles of *RNF213* in angiogenesis regulated by WNT signaling in ECs [26] and in non-mitochondrial oxygen consumption in Her2+ breast cancer cells [27]. These molecular pathways should be investigated in *RNF213* R4810K-induced reduced angiogenesis under hypoxic conditions.

Overexpression of *RNF213* R4810K reduced angiogenesis and mitotic abnormalities; therefore, stimuli that regulate *RNF213* expression are likely to play an important role in MMD etiology as environmental factors. Recently, two studies reported that *RNF213* is upregulated by interferon (IFN) and pro-inflammatory cytokines [11, 21], suggesting the importance of inflammatory signals as environmental factors. Kobayashi et al. [11] demonstrated that INF-β and INF-γ increased *RNF213* expression in HUVECs. IFN-β increased *RNF213* expression levels in an EC-specific manner, whereas IFN-γ did not. *RNF213* upregulation by INF-β was mediated by a STAT binding site located in the promoter of *RNF213*. *RNF213* upregulation by INF-β, which has an anti-angiogenic effect [28], was consistently accompanied by reduced angiogenesis, and *RNF213* suppression using RNA interference partially rescued the IFN-β anti-angiogenic action (Fig. 6.3). Okubo et al. [21] also showed that *RNF213* transcription was strongly activated by synergistic effects of INF-γ and TNF-α in vitro and in vivo. They revealed that AKT and PKR are two major upstream regulators of cytokine-induced *RNF213* expression in ECs. These studies indicated that *RNF213* is a key mediator downstream of the IFN-signaling pathway in ECs. IFN production by infection and/or inflammation could

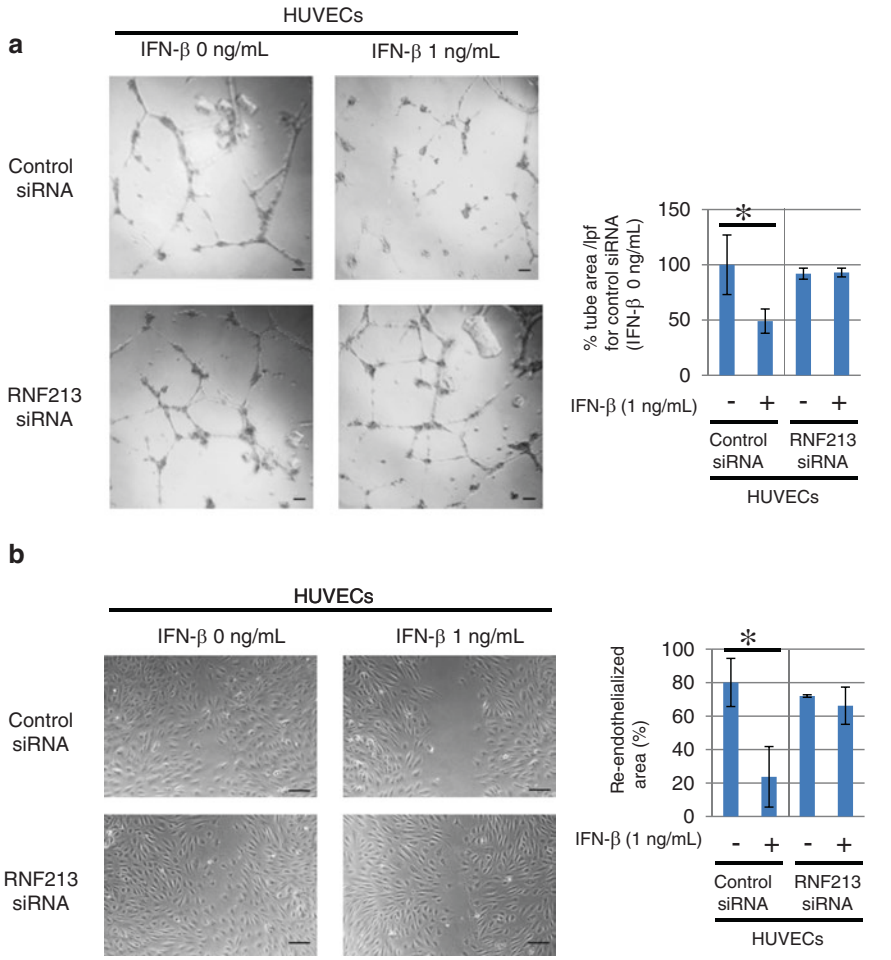

Fig. 6.3 Effects of *RNF213* depletion on IFN-β anti-angiogenic activity in HUVECs (reproduced from [11]). (**a**) Tube formation assays for HUVECs cultured with IFN-β after control or *RNF213* siRNA transfection. Treatment with 0 ng/mL IFN-β after control siRNA transfection was used as a positive control (100%). The scale bars indicate 100 μm. Representative images are shown in the *left panel*. The tube area was quantified by image analysis (*right panel*). A column with a bar represents the mean ± SD (n = 3). **P* < 0.05, according to Student's t-test compared with 0 ng/mL IFN-β within the same siRNA treatment paradigm. (**b**) Migration assays for HUVECs treated with IFN-β (1 ng/mL) after control or *RNF213* siRNA transfection. Treatment with 0 ng/mL IFN-β after control siRNA transfection served as the control. The scale bars indicate 100 μm. Representative images are shown in the *left panel*. The re-endothelialized areas were quantified by image analysis (*right panel*). A column with a bar represents mean ± SD (n = 3). **P* < 0.05, according to Student's t-test compared with 0 ng/mL IFN-β within the same siRNA treatment paradigm

to be related to MMD etiology through *RNF213* upregulation, which is supported by association between MMD and type I interferonopathies (Aicardi-Goutieres syndrome) [29].

6.4 Conclusions

Cerebrovascular stenotic changes and moyamoya vessel formation, as seen in MMD, have not been observed in *Rnf213* KO mice under either physiological or stress conditions [7, 8, 10]. In contrast, thinning of the CCA intima and medial layers in *Rnf213*KO mice was observed after CCA ligation [8], which might represent the constrictive arterial remodeling recently reported as a very early morphological change in MMD [13]. Furthermore, *Rnf213* ablation improved the diabetic phenotype in an ER stress-associated diabetic model mouse by protecting pancreatic β cells from apoptosis, suggesting involvement of *RNF213* in UPR, including ERAD [7]. On the other hand, EC-specific *Rnf213* R4810K-equivalent Tg mice exposed to hypoxia showed suppression of responsive cerebral angiogenesis [11], which is consistent with the lowered angiogenesis found in MMD iPSECs and RNF213 R4810K-overexpressing ECs [20]. In addition, cell biological studies demonstrated mitotic dysfunction caused by *RNF213* R4810K [22] or INF-induced upregulation of *RNF213* [11, 21]. These results support the hypothesis that RNF213 R4810K overexpression in ECs, which could result from inflammation/infection, may induce EC dysfunction and cell division abnormalities, leading to MMD onset. Collectively, there is an obvious conflict between the *RNF213* KO and *RNF213* R4810K overexpression models. The former assumes R4810K to be a loss-of-function mutation and the latter does not postulate any genetic assumption. It is still undetermined which of the *Rnf213* KO or Tg mouse model is more appropriate as an MMD model. Further efforts to reproduce MMD-like vascular changes, such as stenosis, in animal models are required.

References

1. Koizumi A. Genetic analysis of familial moyamoya. Annual report of the Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) by Science Research Grants of Ministry of Health, Labor and Welfare, Japan (Chaired by N. Hashimoto); 2010.
2. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A et al. A genome-wide association study identifies *RNF213* as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40. doi:jhg2010132 [pii] 1038/jhg.2010.132.
3. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T et al. Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development. PloS one. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542 PONE-D-10-04031 [pii].

4. Morito D, Nishikawa K, Hoseki J, Kitamura A, Kotani Y, Kiso K, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. Sci Rep. 2014;4:4442. doi:10.1038/srep04442.
5. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of moyamoya disease and associated health risks explored through *RNF213*. Environ Health Prev Med. 2015; doi:10.1007/s12199-015-0498-7.
6. Kotani Y, Morito D, Yamazaki S, Ogino K, Kawakami K, Takashima S, et al. Neuromuscular regulation in zebrafish by a large AAA+ ATPase/ubiquitin ligase, mysterin/RNF213. Sci Rep. 2015;5:16161. doi:10.1038/srep16161.
7. Kobayashi H, Yamazaki S, Takashima S, Liu W, Okuda H, Yan J et al. Ablation of *Rnf213* retards progression of diabetes in the Akita mouse. Biochemical and biophysical research communications. 2013;432(3):519–25. doi:10.1016/j.bbrc.2013.02.015 S0006-291X(13)00238–6 [pii].
8. Sonobe S, Fujimura M, Niizuma K, Nishijima Y, Ito A, Shimizu H 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. doi:10.1016/j.brainres.2014.01.011.
9. Sonobe S, Fujimura M, Niizuma K, Fujimura T, Furudate S, Nishijima Y, et al. Increased vascular MMP-9 in mice lacking *RNF213*: moyamoya disease susceptibility gene. Neuroreport. 2014;25(18):1442–6. doi:10.1097/WNR.0000000000000289.
10. Ito A, Fujimura M, Niizuma K, Kanoke A, Sakata H, Morita-Fujimura Y, et al. Enhanced post-ischemic angiogenesis in mice lacking *RNF213*; a susceptibility gene for moyamoya disease. Brain Res. 2015;1594:310–20. doi:10.1016/j.brainres.2014.11.014.
11. Kobayashi H, Matsuda Y, Hitomi T, Okuda H, Shioi H, Matsuda T, et al. Biochemical and functional characterization of *RNF213* (*Mysterin*) R4810K, a susceptibility mutation of moyamoya disease, in angiogenesis in vitro and in vivo. J Am Heart Assoc. 2015;4(7) doi:10.1161/JAHA.115.002146.
12. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, et al. Genetics and biomarkers of Moyamoya disease: significance of RNF213 as a susceptibility gene. J Stroke. 2014;16(2):65–72. doi:10.5853/jos.2014.16.2.65.
13. Kaku Y, Morioka M, Ohmori Y, Kawano T, Kai Y, Fukuoka H, et al. Outer-diameter narrowing of the internal carotid and middle cerebral arteries in moyamoya disease detected on 3D constructive interference in steady-state MR image: is arterial constrictive remodeling a major pathogenesis? Acta Neurochir. 2012;154(12):2151–7. doi:10.1007/s00701-012-1472-4.
14. Yoshioka M, Kayo T, Ikeda T, Koizumi A. A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes. 1997;46(5):887–94.
15. Wang J, Takeuchi T, Tanaka S, Kubo SK, Kayo T, Lu D, et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. J Clin Invest. 1999;103(1):27–37. doi:10.1172/JCI4431.
16. Martin-Gronert MS, Ozanne SE. Metabolic programming of insulin action and secretion. Diabetes Obes Metab. 2012;14(Suppl 3):29–39. doi:10.1111/j.1463-1326.2012.01653.x.
17. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. Science. 2011;334(6059):1081–6. doi:10.1126/science.1209038.
18. Liu M, Hodish I, Rhodes CJ, Arvan P. Proinsulin maturation, misfolding, and proteotoxicity. Proc Natl Acad Sci U S A. 2007;104(40):15841–6. doi:10.1073/pnas.0702697104.
19. Allen JR, Nguyen LX, Sargent KE, Lipson KL, Hackett A, Urano F. High ER stress in beta-cells stimulates intracellular degradation of misfolded insulin. Biochem Biophys Res Commun. 2004;324(1):166–70. doi:10.1016/j.bbrc.2004.09.035.
20. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K 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(1):13–9. doi:10.1016/j.bbrc.2013.07.004 S0006-291X(13)01144–3 [pii].
21. Ohkubo K, Sakai Y, Inoue H, Akamine S, Ishizaki Y, Matsushita Y, et al. Moyamoya disease susceptibility gene RNF213 links inflammatory and angiogenic signals in endothelial cells. Sci Rep. 2015;5:13191. doi:10.1038/srep13191.
22. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K et al. The moyamoya disease susceptibility variant RNF213 R4810K (rs112735431) induces genomic instability by mitotic abnormality. Biochem Biophy Res Commun. 2013;439(4):419–26. doi:10.1016/j.bbrc.2013.08.067 S0006-291X(13)01414–9 [pii].
23. Lucke T, Clewing JM, Boerkoel CF, Hartmann H, Das AM, Knauth M, et al. Cerebellar atrophy in Schimke-immuno-osseous dysplasia. Am J Med Genet A. 2007;143A(17):2040–5. doi:10.1002/ajmg.a.31878.
24. Bober MB, Khan N, Kaplan J, Lewis K, Feinstein JA, Scott Jr CI, et al. Majewski osteodysplastic primordial dwarfism type II (MOPD II): expanding the vascular phenotype. Am J Med Genet A. 2010;152A(4):960–5. doi:10.1002/ajmg.a.33252.
25. Codd PJ, Scott RM, Smith ER. Seckel syndrome and moyamoya. J Neurosurg Pediatr. 2009;3(4):320–4. doi:10.3171/2008.12.PEDS08205 [pii] 31.3171/2008.12.PEDS08205.
26. Scholz B, Korn C, Wojtarowicz J, Mogler C, Augustin I, Boutros M, et al. Endothelial RSPO3 controls vascular stability and pruning through non-canonical WNT/Ca(2+)/NFAT signaling. Dev Cell. 2016;36(1):79–93. doi:10.1016/j.devcel.2015.12.015.
27. Banh RS, Iorio C, Marcotte R, Xu Y, Cojocari D, Rahman AA, et al. PTP1B controls non-mitochondrial oxygen consumption by regulating RNF213 to promote tumour survival during hypoxia. Nat Cell Biol. 2016;18(7):803–13. doi:10.1038/ncb3376.
28. Takano S, Ishikawa E, Matsuda M, Yamamoto T, Matsumura A. Interferon-beta inhibits glioma angiogenesis through downregulation of vascular endothelial growth factor and upregulation of interferon inducible protein 10. Int J Oncol. 2014;45(5):1837–46. doi:10.3892/ijo.2014.2620.
29. Ramesh V, Bernardi B, Stafa A, Garone C, Franzoni E, Abinun M, et al. Intracerebral large artery disease in Aicardi-Goutieres syndrome implicates SAMHD1 in vascular homeostasis. Dev Med Child Neurol. 2010;52(8):725–32. doi:10.1111/j.1469-8749.2010.03727.x.

Chapter 7
Pathological Investigation on *RNF213*: Animal Models of *Rnf213*-Knockout and Knock-in Mice

Miki Fujimura, Shigeo Kure, and Teiji Tominaga

Abstract Moyamoya disease (MMD) is a rare cerebrovascular disease characterized by a progressive stenosis at the terminal portion of the internal carotid artery and an abnormal vascular network at the base of the brain. Although its etiology is undetermined, recent genome-wide and locus-specific association studies identified *RNF213* as an important susceptibility gene for MMD among East Asian population. A polymorphism in c.14576G>A in *RNF213* was evident in almost all of the familial patients with MMD and 80% of sporadic cases, but the exact role of *RNF213* polymorphism in the development of MMD remains unknown. To answer this question, we generated genetically engineered mice lacking *Rnf213* by homologous recombination (*Rnf213*-knockout mice), as well as *Rnf213*-knock-in mice expressing a missense mutation in mouse *Rnf213*, p. R4828K, corresponding to human *RNF213*, p. R4859K, in MMD patients. Although both mice did not spontaneously develop MMD up to 64 weeks of age as shown by high-resolution magnetic resonance angiography and carbon black injection analysis of the circle of Willis, the biological response of these mice under a variety of insults, such as ischemia or immunological stimuli, provided new insights for the pathogenesis of this rare entity.

Keywords Moyamoya disease • Genetics • *RNF213* • Susceptibility gene

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7.1 Introduction

Moyamoya disease (MMD) is characterized by a progressive stenosis at the terminal portion of the internal carotid artery (ICA) and an abnormal vascular network at the base of the brain [1, 2]. The etiology of MMD remains unknown, but the role of genetic factors had been implicated in the etiology of this rare entity. Previous studies suggested several loci associated with MMD: 3p24–p26 [3], 6q25 [4], 8q23 [5], and 17q25 [6]. More recently, the RING finger protein 213 gene (*RNF213*) in the 17q25-ter region was identified as a novel susceptibility gene for MMD [7–9]. We have reported that a single nucleotide polymorphism (SNP) in c.14576G>A in *RNF213* was identified in 100% of familial MMD patients and 79% of sporadic cases [7]. This SNP of *RNF213* was also found to be associated with the earlier onset and severer forms of MMD, suggesting its value as a favorable biomarker for predicting prognosis [10]. But the mechanism underlying the *RNF213* polymorphism leads to MMD remains unclear, thus we sought to answer this question by generating *Rnf213*-knockout mice (*Rnf213−/−*) [11] and *Rnf213*-knock-in mice [12]. In this chapter, we focus on the pathophysiology of MMD elucidated by the most recent observations of these *Rnf213* mutant mice.

7.2 Generation of *Rnf213*-Deficient Mice (*Rnf213 −/−*) and Their Phenotype

The conventional knockout mice of *Rnf213* were generated by deleting the exon 32, the largest exon of this gene [11] (Fig. 7.1). The targeting vector was produced by cloning a loxP site into the 5′ site of exon 32 and a fragment containing a FRT-flanked neomycin resistance gene and a loxP site into the 3′ site of exon 32. The gene construct was linearized and electroporated into C57BL/6 ES cells and selected with G418 and ganciclovir. Correctly targeted clones were injected into Balb/c blastocysts to generate chimeric mice with the targeted allele incorporated into the germ lines. The chimeric male mice were mated with female C57BL/6 mice, and germ line transmission of the targeted allele was examined in the offspring. Offspring carrying the target allele were bred with transgenic C57BL/6 mice expressing Flp recombinase gene under the control of the CAG promoter to generate mice carrying the floxed allele, which could be bred for conditional gene targeting. Offspring carrying the floxed allele were bred with transgenic C57BL/6 mice expressing the Cre recombinase gene under the control of the CAG promoter to generate mice heterozygous for the *Rnf213* deficiency (*RNF213 −/+*) by the deletion of exon 32. Heterozygous male and female mice were bred to produce homozygous offspring (*Rnf213 −/−*). Genotyping was performed by polymerase chain reaction (PCR) using specific primers to exon 32 [11].

As a result, *Rnf213−/−* were born and grew normally (Fig. 7.1), and complete removal of *Rnf213* exon 32 from genomic DNA was confirmed by PCR [11]. Then we subjected these mice to time sequential analysis by magnetic resonance

Fig. 7.1 General appearance of *Rnf213*-knockout mice (*Rnf213−/−*) and its gene construct. Conventional knockout mice were generated by the Cre-lox system by deleting Exon 32 of *Rnf213*

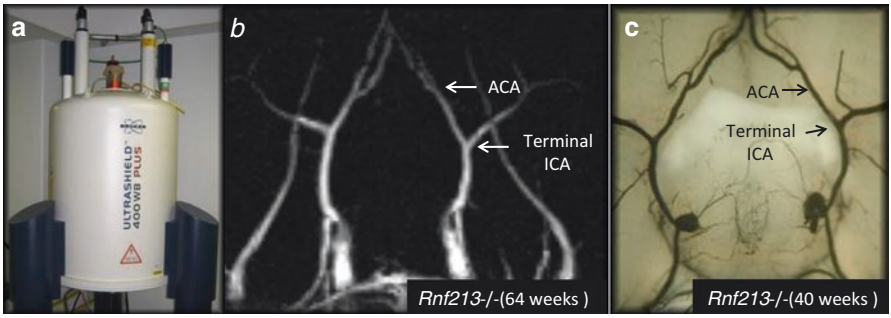

Fig. 7.2 (**a**) The 9.4 tesla high-resolution magnetic resonance (MR) system. (**b**) MR angiography finding of *Rnf213−/−* at 64 weeks of age, indicating the absence of characteristic findings of MMD. (**c**) Microscopic view of the base of the brain at 40 weeks demonstrated no steno-occlusive changes in the vascular structure of the circle of Willis in *Rnf213−/−*

angiography (MRA) using a dedicated small animal scanner with a 5 cm bore operating at 9.4 tesla field strengths (AV400WV, Bruker BioSpin) [11] (Fig. 7.2a). The *Rnf213 −/−* did not spontaneously develop MMD, and there were no characteristic angio-architectures mimicking MMD by MRA up to 64 weeks of age [11] (Fig. 7.2b). The anatomy of the circle of Willis was further evaluated by a trans-cardiac injection of carbon black dye in *Rnf213−/−*, but there were no characteristic findings of MMD up to 64 weeks of age [11] (Fig. 7.2c). Taken together, target disruption of *Rnf213* in knockout mice did not sufficiently induce MMD-like morphology.

7.3 Generation of *Rnf213*-Knock-in Mice and Their Phenotype

The *Rnf213* gene was modified to generate *Rnf213* R4828K mice using a gene-targeting approach such that exon 61 contained the human *RNF213* R4859K mutation [12]. In the targeting vector, the original AGA triplet codon 4828 was changed to AAA by site-directed mutagenesis, creating the R4828K mutation corresponding to the human *RNF213*, R4859K mutation. A neomycin cassette flanked by LoxP sites was introduced upstream of exon 61. C57BL/6 embryonic stem cells were electroporated, and positive clones were screened for homologous recombination by a Southern blot analysis. Integration of the targeting vector into the mouse genome by homologous recombination was confirmed in a targeted Bac clone by direct sequencing of the PCR products [12]. Correctly targeted embryonic stem cells were injected into Balb/c blastocysts to create chimerical animals. Chimeric mice were mated with C57BL/6 mice, and offspring coated with black hair were selected. Offspring that were heterozygous for the R4828K+Neo allele were crossed with CAG-Cre delete mice to excise the neomycin cassette. *Rnf213*-knock-in mice in which the neomycin cassette was successfully deleted were backcrossed with C57BL/6 wild-type mice to excise the Cre transgene. Heterozygous Cre transgene-deleted *Rnf213*-knock-in male and female mice were bred to produce homozygous offspring (homozygous Cre transgene-deleted *Rnf213*-knock-in mice).

As a result, homozygous mutant and heterozygous mutant mice were born and grew normally [12]. The *Rnf213*-knock-in mice did not spontaneously develop MMD, and there were no characteristic angio-architectures of MMD by MRA up to 64 weeks of age [12] (Fig. 7.3a). We further evaluated the vascular anatomy of the

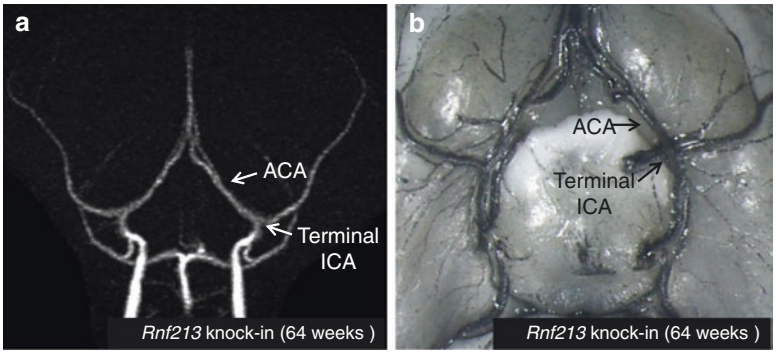

Fig. 7.3 (**a**) MR angiography finding of *Rnf213*-knock-in mice at 64 weeks of age, indicating the absence of characteristic findings of MMD. (**b**) Microscopic view of the base of the brain at 64 weeks demonstrated no steno-occlusive changes in the vascular structure of the circle of Willis in knock-in mice

circle of Willis by a trans-cardiac injection of DiI, but there were no characteristic findings of MMD up to 64 weeks of age [12]. No steno-occlusive changes were detected at the terminal portion of the ICA, without any development of the abnormal vascular network at the base of the brain (Fig. 7.3b).

7.4 Explanation Why *Rnf213*-Mutant Mice Did Not Spontaneously Develop MMD

The reasons why both *Rnf213−/−* and *Rnf213*-knock-in mice did not spontaneously develop MMD could be explained as follows. Firstly, it is has not yet been established whether the *RNF213* polymorphism in patients with MMD is a loss-of-function or gain-of-function mutation. If the SNP of *RNF213* is a gain-of-function mutation in patients with MMD, it is natural that we failed to induce MMD in *Rnf213−/−*, having a loss-of function mutation of *Rnf213*. In fact, tube formation in induced pluripotent stem cells (iPSCs) derived from the vascular endothelial cells collected from MMD patients with R4810K in *RNF213* were downregulated [13], and the overexpression of *RNF213* R4810K, mimicking SNP in patients with MMD, in endothelial cells was reported to result in the suppression of angiogenesis [14]. These findings together indicate the characteristic polymorphism in *RNF213* could be a gain-of-function mutation. Alternatively, zebrafish lacking *RNF213* through the knockdown of *RNF213* showed abnormal sprouting vessels in head lesions, particularly from the optic vessels, suggesting that *RNF213* could be a loss-of-function mutation [8].

Another possible explanation why the *Rnf213* mutant mice did not spontaneously develop MMD is that a *RNF213* mutation in MMD patients was not sufficient to induce the phenotype of MMD, and additional environmental factors in addition to genetic factor were needed for the development of MMD. This observation is in accordance with the fact that the characteristic SNPs of *RNF213* were found among 2% of the general Japanese population [8], but the prevalence of MMD is as low as 6.03 per 100,000 population [15], indicating the very low penetrance rate of MMD. Therefore, we speculated that not only *RNF213* polymorphisms but also additional environmental factors are necessary to induce MMD [16]. Thus, we conceived to load a variety of insults such as ischemia or immunological adjuvant administration to *Rnf213* mutant mice in the subsequent experiments. The demographic view of the possible mechanism underlying the development of MMD is shown in Fig. 7.4. The results of our in vivo experiment using *Rnf213*-knockout mice subjected to a variety of insults were summarized in Table 7.1.

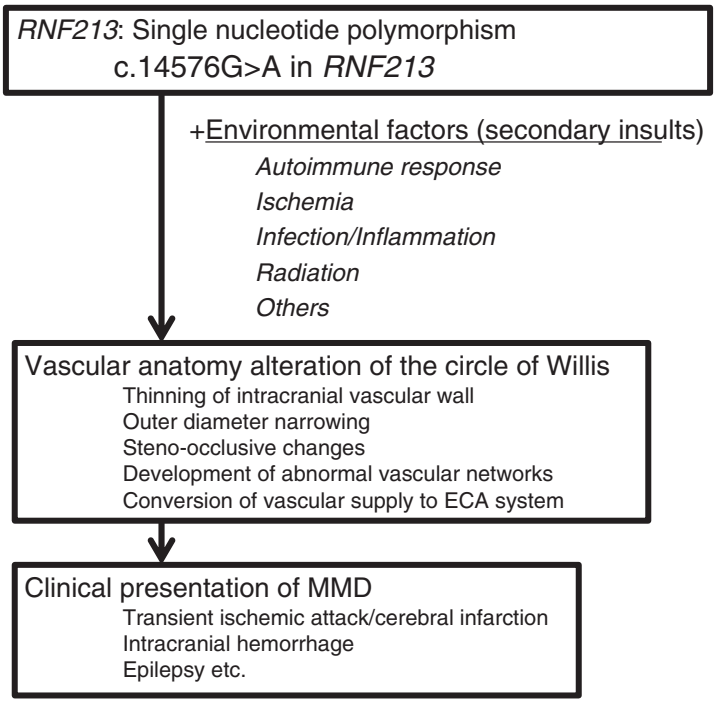

Fig. 7.4 Demographic view of the possible mechanism underlying the development of MMD. *ECA* external carotid artery

Table 7.1 Results of the in vivo experiment using *Rnf213*-knockout mice subjected to a variety of insults

Insults (stimuli)	Evaluation items	Timing after the insults	Results in *Rnf213−/−*
CCA ligation	Wall structure	14 days	Altered remodeling pattern
	Vascular MMP-9	1 day, 7 days	Increased vascular MMP-9
MCAO	Infarction volume	48 hours	No difference
	Angiogenesis (CD31)	28 days	No difference
Femoral artery ligation	Angiogenesis (CD31)	28 days	Enhanced
	Blood flow (hind limb)	28 days	Increased
	Ambulatory impairment	7 days	Improved
Immunological adjuvant administration	Regulatory T cell ratio	28 days	Decreased

CCA common carotid artery, *MCAO* middle cerebral artery occlusion, *MMP* matrix metalloproteinase, *RNF213−/− Rnf213*-knockout mice

7.5 Vascular Remodeling and Angiogenesis Under Ischemic Insults in *Rnf213−/−*

7.5.1 Altered Vascular Remodeling Pattern After Common Carotid Artery (CCA) Ligation in Rnf213−/−

We also evaluated the histopathological characteristics of the vascular wall structure and showed no apparent abnormality in *Rnf213−/−* including intimal hyperplasia or medial layer thinness, both of which are the characteristic findings of MMD. Thus, we employed a common CCA ligation model, which reproducibly induces arterial wall hyperplasia [17]. After CCA ligation, wild-type mice showed temporary hyperplasia of the intima and medial layers 14 days after the insult, but *Rnf213−/−* exhibited different remodeling pattern [11]. The vascular wall structures including medial layer were significantly thinner in *Rnf213−/−* 14 days after CCA ligation [11], which could partly explain the early characteristic change of MMD including vascular wall thinning as shown by high-resolution magnetic resonance imaging (MRI) in MMD patients [18]. In fact, immune-histochemical analysis of the alternative section revealed increased expression of matrix metalloproteinase (MMP)-9 in *Rnf213−/−* after CCA ligation [19], which is consistent with the increased serum expression of MMP-9 in patients with MMD [20].

7.5.2 Angiogenesis After Middle Cerebral Artery Occlusion (MCAO) and Hind Limb Ischemia in Rnf213−/−

To evaluate angiogenic activity in the chronic state after transient MCAO, we evaluated the vascular density in the cerebral parenchyma by immunohistochemistry using anti-CD31 antibody 28 days after 90 minutes MCAO [21]. The mortality rates in *Rnf213−/−* and wild-type mice were both 50%. No significant difference was observed in cerebral parenchymal vascular density as indicated by CD31-positive cells between wild-type mice and *Rnf213−/−* [21].

Systemic angiogenesis after hind limb ischemia was also evaluated by immunohistochemistry in the gastrocnemius (GC) muscle with the anti-CD31 antibody 28 days after the induction of ischemia. We found that the density of CD31-positive cells was significantly higher in *Rnf213 −/−* than in wild-type mice, indicating that angiogenesis in the GC muscle was enhanced more in *Rnf213 −/−* after chronic hind limb ischemia [21]. Accordingly, the ambulatory impairment score was significantly improved in *Rnf213 −/−* than in wild-type mice 3 and 7 days after chronic hind limb ischemia [21], indicating the increased angiogenesis and improved functional outcome in *Rnf213−/−* after chronic hind limb ischemia.

Taken together with the result of CCA ligation model, *Rnf213−/−* exhibited vascular wall thinning and increased angiogenic activity after chronic ischemia. These results could partly reflect the characteristic feature of MMD.

7.6 Decreased Ratio of Regulatory T Cells After Immunological Adjuvant Administration to *Rnf213−/−*

7.6.1 Rationale of the Immunological Stimuli as Supplemental Insult in Addition to Rnf213 Mutation

While considering the role of environmental factors in addition to the *RNF213* abnormality in MMD, it should be noted that higher RNF213 mRNA levels were detected particularly in human immune tissues, such as the spleen, leukocytes, and lymph nodes [7]. Furthermore, autoimmune responses have been implicated in the pathophysiology of MMD among East Asian populations [22, 23], among which the prevalence of the *RNF213* polymorphism is higher than the other races. Based on these findings, it is conceivable that immunological reactions are one of the strongest candidates for a secondary insult in addition to genetic factors in the pathogenesis of MMD (Fig. 7.4). Therefore, we sought to expose *Rnf213−/−* to various immunological adjuvants including muramyl dipeptide (MDP)-Lys (L18) or complete Freund's adjuvant (CFA) and determined whether they developed MMD by evaluating the role of an immunological stimulation as a supplementary insult to the target disruption of *Rnf213* in the pathophysiology of MMD [24].

7.6.2 Failure of MMD Induction in Rnf213−/− After Immunological Adjuvant Administration

MRA was performed continuously in the same mouse administered MDP-Lys (L18) or complete Freund's adjuvant (CFA) as the immunological adjuvant up to 40 weeks of age [24]. MDP-Lys (L18) is a peptide present in the bacterial cell wall, is a nucleotide-binding oligomerization domain (NOD)-2 agonist that controls the activation of the transcription factor NF-κB, and has been implicated in a number of autoimmune reactions. CFA was composed of inactivated and dried *M. tuberculosis*. As a result, there were no characteristic angio-architecture mimicking MMD, such as bilateral steno-occlusive changes in the terminal portion of the ICA, and an abnormal vascular network did not develop at the base of the brain in mice up to 40 weeks of age after the immunological adjuvant administration. No significant difference was observed in MRA findings or the anatomy of the circle of Willis between wild-type mice and *Rnf213−/−* after the administration of MDP-Lys (L18). There was also no characteristic finding of MMD, and no significant difference was noted in MRA findings or the anatomy of the circle of Willis between wild-type mice and *Rnf213*-deficient mice up to 40 weeks after the administration of CFA.

7.6.3 *Decreased Ratio of Regulatory T Cells After Immunological Adjuvant Administration to* Rnf213−/−

To evaluate the immunological conditions in *Rnf213−/−* after the immunological adjuvant administration, we measured the weight of the spleen and performed flow cytometry on splenocytes for CD4, CD25, and Foxp3. The weight of the spleen slightly increased 4 weeks after the administration of immunological adjuvants, which confirmed appropriate immunological responses. Interestingly, the ratio of regulatory T cells was significantly lower in *Rnf213−/−* than in wild-type mice 4 weeks after the administration of MDP-Lys (L18) [24]. These results were confirmed by an immune-histochemical analysis of Foxp3 in the spleen 4 weeks after the administration of immunological adjuvants [24]. In order to further evaluate the ability to produce regulatory T cells, we administered PC61, a CD25 antibody that induces the depletion of Foxp3-positive cells, to both animals twice a week for 4 weeks and then performed flow cytometry. The ratio of regulatory T cells was significantly lower in *Rnf213−−/−* than in wild-type mice after the administration of PC61 [24]. These results suggested the potential role of *RNF213* in the differentiation of regulatory T cells. Taken together with the predominant expression of *RNF213* in human immune tissues [7], it is conceivable that the *RNF213* abnormality may compromise immunological self-tolerance, thereby contributing to the development of MMD in the patients [24]. The demographic view of our hypothesis is shown in Fig. 7.5. Further evaluation of immune status in patients with MMD is necessary to address this important issue.

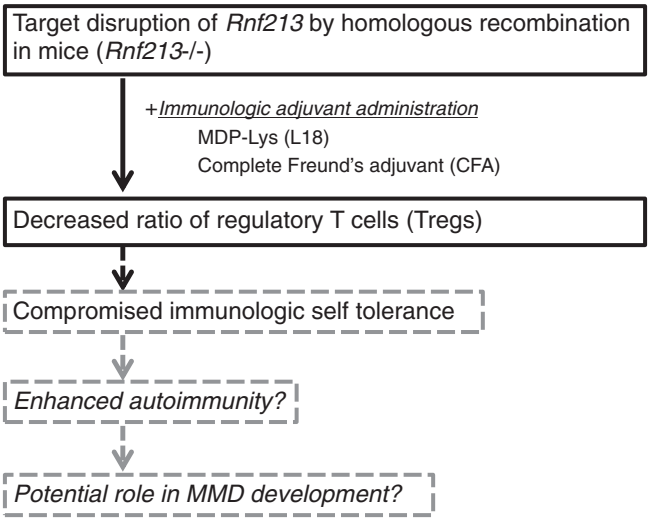

Fig. 7.5 Demographic view of our hypothesis: possible role of *Rnf213* deficiency in autoimmunity and its involvement in the development of MMD. *MDP* muramyl dipeptide

7.7 Conclusion

To evaluate the potential role of *RNF213* in the development of MMD, we generated both *Rnf213*-deficient mice and *Rnf213*-knock-in mice expressing a missense mutation in mouse *Rnf213*, p. R4828K, corresponding to human *RNF213*, p. R4859K, in MMD patients. Although both mice did not spontaneously develop MMD up to 64 weeks of age as shown by the 9.4 tesla MRA, the biological response of these mice under a variety of insults, such as ischemia or immunological stimuli (Table 7.1), provided new insights for the pathogenesis of this rare entity.

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. Research Committee on the Pathology and Treatment of Spontaneous Occlusion of the Circle of Willis, Health Labour Sciences Research Grant for Research on Measures for Infractable Diseases. Guidelines for diagnosis and treatment of moyamoya disease (Spontaneous Occlusion of the Circle of Willis). Neurol Med Chir (Tokyo). 2012;52:245–66.
3. Ikeda H, Sasaki T, Yoshimoto T, Fukui M, Arinami T. Mapping of a familial moyamoya disease gene to chromosome 3p24.2-p26. Am J Hum Genet. 1999;64:533–7.
4. Inoue TK, Ikezaki K, Sasazuki T, Matsushima T, Fukui M. Linkage analysis of moyamoya disease on chromosome 6. J Child Neurol. 2000;15:179–82.
5. Sakurai K, Horiuchi Y, Ikeda H, Ikezaki K, Yoshimoto T, Fukui M, et al. A novel susceptibility locus for moyamoya disease on chromosome 8q23. J Hum Genet. 2004;49:278–81.
6. Yamauchi T, Tada M, Houkin K, Tanaka T, Nakamura Y, Kuroda S, et al. Linkage of familial moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. Stroke. 2000;31:930–5.
7. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56:34–40.
8. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6:e22542.
9. Wu Z, Jiang H, Zhang L, Xu X, Zhang X, Kang Z, et al. Molecular analysis of RNF213 gene for moyamoya disease in the Chinese Han population. PLoS One. 2012;7:e48179.
10. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78:803–10.
11. Sonobe S, Fujimura M, Niizuma K, Nishijima Y, Ito A, Shimizu H, et al. Temporal profile of the vascular anatomy evaluated by 9.4-tesla magnetic resonance angiography and histopathological analysis in mice lacking *RNF213*; a susceptibility gene for moyamoya disease. Brain Res. 2014;1552:64–71.
12. Kanoke A, Fujimura M, Niizuma K, Ito A, Sakata H, Sato-Maeda M, 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.
13. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, 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.
14. Kobayashi H, Yamazaki S, Takashima S, Liu W, Okuda H, Yan J, et al. Ablation of Rnf213 retards progression of diabetes in the Akita mouse. Biochem Biophys Res Commun. 2013;432:519–25.
15. Kuriyama S, Kusaka Y, Fujimura M, Wakai K, Tamakoshi K, Hashimoto S, et al. Prevalence and clinicoepidemiological features of moyamoya disease in Japan: findings from a nationwide epidemiological survey. Stroke. 2008;39:42–7.
16. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, et al. Genetics and biomarkers of moyamoya disease: significance of RNF213 as a susceptibility gene. J Stroke. 2014;16:65–72.
17. Godin D, Ivan E, Johnson C, Magid R, Galis ZS. Remodeling of carotid artery is associated with increased expression of matrix metalloproteinases in mouse blood flow cessation model. Circulation. 2000;102:2861–6.
18. Ryoo S, Cha J, Kim SJ, Choi JW, Ki CS, Kim KH, et al. High-resolution magnetic resonance wall imaging findings of Moyamoya disease. Stroke. 2014;45:2457–60.
19. Sonobe S, Fujimura M, Niizuma K, Fujimura T, Furudate S, Nishijima Y, et al. Increased vascular MMP-9 in mice lacking *RNF213*; moyamoya disease susceptibility gene. Neuroreport. 2014;25:1442–6.
20. Fujimura M, Watanabe M, Narisawa A, Shimizu H, Tominaga T. Increased expression of serum matrix metalloproteinase-9 in patients with moyamoya disease. Surg Neurol. 2009;72:476–80.
21. Ito A, Fujimura M, Niizuma K, Kanoke A, Sakata H, Morita-Fujimura Y, et al. Enhanced post-ischemic angiogenesis in mice lacking RNF213; a susceptibility gene for moyamoya disease. Brain Res. 2015;1594:310–20.
22. Kim SJ, Heo KG, Shin HY, Bang OY, Kim GM, Chung CS, et al. Association of thyroid autoantibodies with moyamoya-type cerebrovascular disease: a prospective study. Stroke. 2010;41:173–6.
23. Li H, Zhang ZS, Dong ZN, Ma MJ, Yang WZ, Han C, et al. Increased thyroid function and elevated thyroid autoantibodies in pediatric patients with moyamoya disease: a case-control study. Stroke. 2011;42:1138–9.
24. Kanoke A, Fujimura M, Niizuma K, Fujimura T, Kakizaki A, Ito A, et al. Temporal profile of magnetic resonance angiography and decreased ratio of regulatory T cells after immunological adjuvant administration to mice lacking RNF213, a susceptibility gene for moyamoya disease. Brain Res. 1642;2016:1–9.

Chapter 8
RNF213 and Circulating Factors in Patients with Moyamoya Disease

Oh Young Bang

Abstract Moyamoya disease (MMD) is a unique cerebrovascular disease characterized by the progressive stenosis of large intracranial arteries and a hazy network of basal collaterals. Because the etiology of MMD is unknown, the diagnosis of MMD is based on characteristic angiographic findings, and there is no specific treatment to prevent MMD progression. This review summarizes the recent advances in MMD pathophysiology, including the genetic and circulating factors related to disease development. Recently, the *Ring finger 213 (RNF213)* gene in the 17q25-ter region was identified as the susceptibility gene for MMD in East Asians. Although the exact function of *RNF213* is unknown, the *RNF213* genetic variant may be associated with the changes in circulating factors and the response to environmental factors. Such interactions of the *RNF213* genetic variant with circulating factors or environmental factors may play important roles in the development of the vascular stenosis and aberrant angiogenesis in complex ways. Circulating factors include the related changes in circulating vascular progenitor cells, cytokines related to vascular remodeling and angiogenesis, and endothelium, such as caveolin which is a plasma membrane protein. With a better understanding of MMD pathophysiology, nonsurgical approaches targeting MMD pathogenesis may be available to stop or slow the progression of this disease in the future.

Keywords Angiogenesis • Biomarkers • Cytokines • Growth factors • *RNF213* • Moyamoya disease

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8.1 Introduction

Moyamoya disease (MMD) is a unique cerebrovascular disease characterized by the progressive stenosis of the distal internal carotid artery (ICA) and the resulting hazy network of basal collaterals called moyamoya vessels.

The main pathological changes of the stenotic segment in MMD are the fibrocellular thickening of the intima (e.g., the hyper-proliferation of the vessel wall components, active angiogenesis, and matrix accumulation), irregular undulation of the internal elastic laminae, medial thinness (e.g., an attenuation of media), and a decrease in the outer diameter [1–6]. Recent neuroimaging techniques, such as high-resolution magnetic resonance imaging studies of patients with MMD, have demonstrated a constrictive remodeling (e.g., the narrowing of the arterial outer diameter) and a long-segment concentric enhancement in affected segments and a concentric enhancement of the symptomatic segments [7]. These findings are consistent with the results of previous pathological reports that showed intimal hyperplasia and medial thinness [2, 3]. The complicated pathologic features of the stenotic segments of MMD (e.g., a coexistence of proliferation and shrinkage) and the unknown nature of the neovascularization (e.g., an aberrant vs. compensatory process) suggest that MMD pathophysiology is a complex process.

Owing to the currently limited understanding of MMD, there is no specific biomarker for the diagnosis of MMD or treatment to prevent MMD progression. The criteria for the diagnosis of MMD are based on characteristic angiographic findings. However, the angiographic findings may not be sensitive or specific to MMD. The current diagnostic criteria require the presence of prominent basal collaterals for the diagnosis of MMD, but a decision on the presence of basal collaterals can be subjective. In patients with MMD, the angiographic findings can differ according to the progressive stage and age of presentation, and the characteristic angiographic findings are not consistently observed in all courses of MMD [8–11]. In patients with an early stage of Suzuki's angiographic grading, the abnormal vascular network is not yet evident [12]. In addition, unlike in childhood-onset MMD, the basal collaterals are often not prominent in adult-onset MMD (Fig. 8.1) [10, 11].

The purpose of this review is to summarize the recent advances in MMD pathophysiology, including the genetic and circulating factors related to disease development.

8.2 Genetic Biomarkers of MMD

Approximately 10% of individuals with MMD exhibit a familial occurrence. In addition, MMD is also associated with many genetically transmitted disorders, including neurofibromatosis, Down syndrome, sickle cell anemia, and collagen vascular disease. These findings suggest the importance of genetic factors.

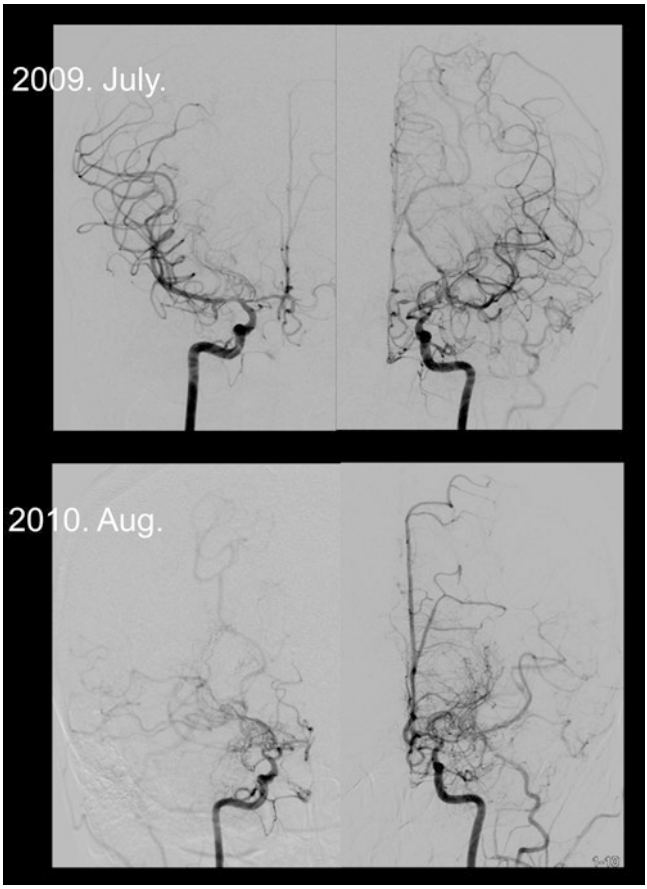

Fig. 8.1 Angiographic progression of MMD in an adult patient with intracranial stenosis (Figure from Bang et al. [10].). A 42-year-old female presented with transient numbness and clumsiness of her left hand. She had mild stenosis on bilateral and proximal middle cerebral arteries. There was no stenosis of the distal internal carotid artery and basal collaterals, called moyamoya vessels, on conventional angiography (*upper lane*). Angiographic findings taken 1 year later show the progression of stenosis and moyamoya vessels bilaterally (*lower lane*). Genetic study revealed *RNF213* mutation associated with MMD (p.Arg4810Lys)

8.2.1 RNF213 as a Susceptible Gene for MMD

The *Ring finger 213* (*RNF213*) gene in the 17q25-ter region was identified as the strongest susceptibility gene for MMD in East Asian people using a genome-wide linkage and exome analysis [13, 14]. The p.R4810K (c.14576G>A) variant of the *RNF213* genetic variant was identified in 95% of patients with familial MMD, 80% with sporadic MMD, and 1.8% of control subjects in a Japanese population [13]. The homozygous p.R4810K variant of *RNF213* predicted an early onset and severe form of MMD in both Japanese [15] and Korean [16] patients with MMD. The

population that is susceptible to MMD, such as carriers of the *RNF213* p.R4810K variant, is estimated to be 16.2 million people in East Asian countries [17]. The number of patients with MMD, which was conservatively estimated at 1 per 300 carriers of the *RNF213* p.R4810K variant, is considered to be 53,800 in East Asian populations [17, 18].

Several susceptibility genes have been identified for MMD and are the basis of disease classification as follows: MMD-2 (gene: *RNF213*), MMD-5 (*ACTA2*), and MMD-6 with achalasia (*GUCY1A3*) [13, 14, 19, 20]. Loci for the disorder have been mapped to chromosome 3p (MMD-1), 8q23 (MMD-3), and Xq28 (MMD-4) [21–23]. Thus, *RNF213* p.Arg4810Lys variant is not the single risk factor for MMD, and other modifying factors, such as other *RNF213* rare variants, another genetic factor, or environmental factors, might provide further insight into the pathogenesis of MMD. Further genetic studies for MMD are warranted, particularly in populations outside East Asia, because the single nucleotide polymorphism (SNP) of p.R4828K in *RNF213* is not the susceptibility gene for MMD in Westerners or South Asian individuals. Novel variants in *RNF213* in non-p.R4828K were recently found in Caucasian and Chinese cases with MMD [14] and in the United States [24]. For example, several variants of *RNF213* in non-p.R4810K (i.e., rs148731719, rs397514563) were recently found in Caucasian and East and South Asian patients with MMD [14, 24–26]. In addition, the clinical manifestations and possibly angiographic findings may differ between Westerners and East Asians [27]. The p.R4810K *RNF213* variant was related to the ischemic type of MMD, while the non-p.R4810K *RNF213* variants, particularly A4399T, were associated with the hemorrhagic type of MMD [25].

8.2.2 RNF213 as a Susceptible Gene for Vascular Injury

The exact function of *RNF213* is unknown. Recent in vivo experiments using genetically engineered *Rnf213* mice addressed the mechanism underlying the *RNF213* SNPs in the development of MMD pathology. The target disruption or missense mutation of *Rnf213* did not induce MMD under normal conditions [28, 29]. These negative results together with the findings of the enhanced postischemic angiogenesis in the mice lacking *Rnf213* after a chronic hind limb ischemia may indicate the importance of environmental factors in addition to the *RNF213* abnormality in the development of abnormal vascular networks in chronic ischemia [30, 31]. There is growing evidence that *RNF213* plays a unique role in endothelial cells regarding the proper gene expression in response to inflammatory signals from the environment [32–34].

In addition to the preclinical data, clinical data has also shown that exposure to environmental factors, such as an autoimmune response and infection/inflammation, in MMD-susceptible subjects may be associated with the angiographic fea-

tures of MMD [30]. For example, autoimmune thyroid disease has been reported in different MMD populations and may be involved in MMD development [35–37]. In addition, the *RNF213* genetic variant may be associated with vascular risk factors, such as hypertension [38], and also could lead to vascular fragility, which may make vessels more vulnerable to hemodynamic stress and secondary insults [30]. Our recent study showed that *RNF213* genetic variant was observed in 21.4% of high-resolution magnetic resonance imaging-confirmed patients with intracranial atherosclerotic stenosis (especially in younger onset), whereas only 1.2% of healthy control subjects had this variant [39]. This finding suggests that *RNF213* is a susceptibility gene not only for MMD but also for intracranial atherosclerotic stenosis in East Asians.

8.3 Circulating Biomarkers of MMD

In addition to genetic biomarkers, there are circulating factors that may be involved in MMD pathogenesis, including circulating vascular progenitor cells, cytokines, caveolin, and microRNAs (miRs).

8.3.1 Circulating Vascular Progenitor Cells

Circulating endothelial progenitor cells (EPCs) that originate from the bone marrow potentially contribute to the neovascularization at the ischemic brain injury site in patients with MMD [40]. In both pediatric and adult patients with MMD, impaired function of circulating EPCs was observed, indicating there is abnormal angiogenesis during MMD pathogenesis [41, 42]. In pediatric patients with MMD, a downregulation of retinaldehyde dehydrogenase 2 (RALDH2) of EPCs was observed, which could contributed to the defective function of EPCs that could be rescued by supplying retinoic acid [43].

In addition to endothelial cells, SMCs (smooth muscle cells) are also involved in this disease process. The MMD pathology is characterized by SMC hyperplasia in the intima. Mutations in SMCs, such as smooth muscle alpha-actin, which is encoded by *ACTA2*, may be involved in the increased proliferation of SMCs, contributing to occlusive diseases [19]. It was also reported that circulating smooth muscle progenitor cells (SPCs) of patients with MMD tended to make more irregularly arranged and thickened tubules, as well as express differential genes compared to that of the healthy controls [44]. These findings suggest a defect in the cell maturation process that might have occurred in the SPCs from MMD patients.

8.3.2 Cytokine and Their Polymorphisms

Various cytokines and their polymorphisms are associated with MMD, including (a) growth factors, such as vascular endothelial growth factor (VEGF); (b) cytokines related to vascular remodeling and angiogenesis, such as matrix metalloproteinases (MMPs) and their inhibitors, hypoxia-inducible factor-1α, and cellular retinoic binding protein-1 (CRABP-1); and (c) cytokines related to inflammation [45–50]. The investigations regarding the role of these factors have been inconclusive.

A genetic study of familial MMD investigated the balance between MMPs and their inhibitors and found that the presence of a certain MMP inhibitor genotype may be a predisposing genetic factor for familial MMD [51]. In addition, the levels of several trophic factors, including VEGF, were increased, but the VEGF receptor levels were decreased in MMD compared to that of controls [46, 52, 53]. Certain VEGF polymorphisms were associated with pediatric MMD and poor collateral vessel formation [48]. However, these findings were not observed in other studies [54]. The induction of pro-inflammation cascades and VEGF expression could be secondary to the infarct rather than part of the primary MMD pathology [55].

Kim and colleagues identified a polypeptide spot, CRABP-1, in cerebrospinal fluid from pediatric patients with MMD using a proteomics analysis [49]. A higher CRABP-1 level in the CSF was associated with a typical bilateral involvement and a decrease in the basal collaterals postoperatively in adult MMD [56]. It has been proposed that the retinoids attenuate growth factor-stimulated SMC migration and proliferation, and CRABP-1 can negatively regulate retinoic acid activity [49]. These findings suggest an important role for retinoid signaling in MMD pathogenesis by controlling the growth factor expression [49].

Although pathological analyses have revealed that the affected vessels do not show any inflammatory changes that lead to occlusion [57], the role of inflammation in the fibrocellular thickening of the intima and the disease pathogenesis are also being investigated. The plasma levels of MMPs, monocyte chemoattractant protein-1, E-selectin, and inflammatory cytokines (interleukin-1β) were higher in patients with MMD compared to those in controls [46, 58].

8.3.3 Caveolin

Caveolae are 50–100 nm cell surface plasma membrane invaginations that are abundant in endothelial cells and play a major role in the regulation of endothelial vesicular trafficking and signal transduction [59]. Caveolin-1, a scaffolding protein of the caveolae plasma membrane, is involved in the pathogenesis of cancers and vascular diseases [59]. Caveolin-1 overexpression enhanced caveolae generation and accelerated the capillary tube formation by nearly threefold, while caveolin-1 downregulation reduced the in vitro and in vivo capillary formation and was associated with pathological angiogenesis [59–61]. Both endothelial nitric oxide synthase and

VEGFR2 co-localized in the caveolae, while caveolin-1 expression was critical for VEGF-induced angiogenesis in an ischemic hind limb model [62] and endothelial nitric oxide-related tumor angiogenesis [63]. One study that used an ischemic hind limb model demonstrated that caveolin-1 was also involved in EPC recruitment from the bone marrow [64].

Our recent study showed that caveolin-1 is a key mediator for MMD [65]. The caveolin-1 serum levels decreased in adult patients with MMD and were markedly decreased in those with the *RNF213* variant. Liu and colleagues showed the differential roles of caveolin-1 during the differential phases of angiogenesis, such as caveolin-1 negatively regulating an earlier phase of angiogenesis (i.e., endothelial cell proliferation), but positively regulating a later phase of angiogenesis (i.e., tube formation) [60]. Collectively, the decreased caveolin-1 levels may result in increased proliferation and decreased stabilization/tube formation in patients with MMD. One study investigated the effects of cerebral ischemia in caveolin-1 knockout mice and demonstrated impaired angiogenesis and increased apoptotic cell death [66]. The elucidation of mechanisms of the caveolin-1-related pathological angiogenesis may pave the way for various therapeutic strategies [64, 67].

8.3.4 MicroRNAs

miRs, small noncoding RNAs (~23 nucleopeptides), negatively regulate the expression of many proteins by altering their gene expression through posttranscriptional repression or mRNA degradation [68]. miRs may play an essential role in the regulation of proliferation, differentiation, survival, and aging of various tissues and cells, including vessels and stem cells [69]. A genome-wide miR array analysis of the serum from patients with MMD showed elevated serum levels of miRs associated with *RNF213* and *BRCC3* (i.e., BRCA1/BRCA2-containing complex, an important angiogenesis-related protein), both of which are involved in MMD pathogenesis [70]. In addition, a SNP of miR-196a was associated with MMD [71]. *ANXA1*, which is expressed in endothelium and SMC [72], is a gene target of miR196a and mediates the apoptosis and inhibition of cell proliferation [73].

8.4 Conclusion and Perspectives

Although the pathogenic mechanisms of MMD are still unknown, there is growing evidence that genetic factors and related changes in circulating factors, as well as environmental factors, may play important roles in complex ways (Fig. 8.2). Further studies are needed because there is no relevant MMD animal model using these factors.

With a better understanding of MMD pathophysiology, nonsurgical approaches targeting MMD pathogenesis may be available to stop or slow the progression of

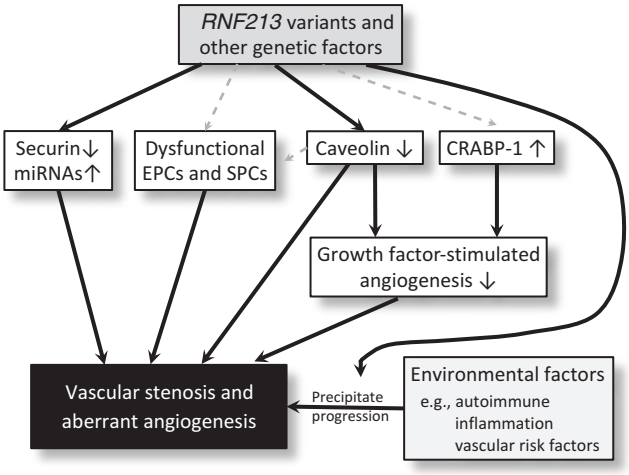

Fig. 8.2 Potential mechanisms of moyamoya disease. The association between genetic, circulating, and environmental factors (Figure modified with permission from Bang et al. [74]. *RNF213 Ring finger 213*, *EPCs* endothelial progenitor cells, *SPCs* smooth muscle progenitor cells, *miRNAs* microRNAs, *CRABP-1* cellular retinoic acid-binding protein-I)

this disease. The possible strategies include targeting growth factors, retinoic acid, caveolin-1, and stem cells. In addition, diagnostic criteria for MMD based on genetic and circulating factors may be available with collaborative works between the clinical hospital bed and the laboratory bench. We have an ongoing prospective follow-up study of patients with MMD involving multimodal biomarkers (NCT2074111 at clinical.trial@gov).

Acknowledgments No conflict of interest to disclose.

References

1. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7:1056–66.
2. Takagi Y, Kikuta K, Nozaki K, Hashimoto N. Histological features of middle cerebral arteries from patients treated for moyamoya disease. Neurol Med Chir (Tokyo). 2007;47:1–4.
3. Oka K, Yamashita M, Sadoshima S, Tanaka K. Cerebral haemorrhage in moyamoya disease at autopsy. Virchows Arch A Pathol Anat Histol. 1981;392:247–61.
4. Chmelova J, Kolar Z, Prochazka V, Curik R, Dvorackova J, Sirucek P, et al. Moyamoya disease is associated with endothelial activity detected by anti-nestin antibody. Biomed Pap Med Fac Univ Palacky Olomouc Czech. 2010;154:159–62.
5. Lin R, Xie Z, Zhang J, Xu H, Su H, Tan X, et al. Clinical and immunopathological features of moyamoya disease. PLoS One. 2012;7:e36386.

6. Takagi Y, Kikuta K, Nozaki K, Fujimoto M, Hayashi J, Imamura H, et al. Expression of hypoxia-inducing factor-1 alpha and endoglin in intimal hyperplasia of the middle cerebral artery of patients with moyamoya disease. Neurosurgery. 2007;60:338–5; discussion 345.
7. Ryoo S, Cha J, Kim SJ, Choi JW, Ki CS, Kim KH, et al. High-resolution magnetic resonance wall imaging findings of moyamoya disease. Stroke. 2014;45:2457–60.
8. Scott RM, Smith ER. Moyamoya disease and moyamoya syndrome. N Engl J Med. 2009;360:1226–37.
9. 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):S238–40.
10. Bang OY, Ryoo S, Kim SJ, Yoon CH, Cha J, Yeon JY, et al. Adult moyamoya disease: a burden of intracranial stenosis in east asians? PLoS One. 2015;10:e0130663.
11. Chung JW, Kim SJ, Bang OY, Kim KH, Ki CS, Jeon P, et al. Determinants of basal collaterals in moyamoya disease: clinical and genetic factors. Eur Neurol. 2016;75:178–85.
12. Suzuki J, Takaku A. Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20:288–99.
13. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies *RNF213*13 as the first moyamoya disease gene. J Hum Genet. 2011;56:34–40.
14. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6:e22542.
15. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576 g>a variant of *RNF213* predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78:803–10.
16. Kim EH, Yum MS, Ra YS, Park JB, Ahn JS, Kim GH, et al. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg. 2016;124:1221–7.
17. Liu W, Hitomi T, Kobayashi H, Harada KH, Koizumi A. Distribution of moyamoya disease susceptibility polymorphism p.R4810k in *RNF213* in east and southeast asian populations. Neurol Med Chir (Tokyo). 2012;52:299–303.
18. Liu W, Hashikata H, Inoue K, Matsuura N, Mineharu Y, Kobayashi H, et al. A rare asian founder polymorphism of raptor may explain the high prevalence of moyamoya disease among east asians and its low prevalence among caucasians. Environ Health Prev Med. 2010;15:94–104.
19. Guo DC, Papke CL, Tran-Fadulu V, Regalado ES, Avidan N, Johnson RJ, 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.
20. Herve D, Philippi A, Belbouab R, Zerah M, Chabrier S, Collardeau-Frachon S, et al. Loss of alpha1beta1 soluble guanylate cyclase, the major nitric oxide receptor, leads to moyamoya and achalasia. Am J Hum Genet. 2014;94:385–94.
21. Ikeda H, Sasaki T, Yoshimoto T, Fukui M, Arinami T. Mapping of a familial moyamoya disease gene to chromosome 3p24.2-p26. Am J Hum Genet. 1999;64:533–7.
22. Sakurai K, Horiuchi Y, Ikeda H, Ikezaki K, Yoshimoto T, Fukui M, et al. A novel susceptibility locus for moyamoya disease on chromosome 8q23. J Hum Genet. 2004;49:278–81.
23. Janczar S, Fogtman A, Koblowska M, Baranska D, Pastorczak A, Wegner O, et al. Novel severe hemophilia a and moyamoya (sham) syndrome caused by xq28 deletions encompassing f8 and brcc3 genes. Blood. 2014;123:4002–4.
24. Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, et al. *RNF213* rare variants in an ethnically diverse population with moyamoya disease. Stroke. 2014;45:3200–7.
25. Wu Z, Jiang H, Zhang L, Xu X, Zhang X, Kang Z, et al. Molecular analysis of *RNF213*gene for moyamoya disease in the chinese han population. PLoS One. 2012;7:e48179.

26. Ma J, Liu Y, Ma L, Huang S, Li H, You C. *RNF213* polymorphism and moyamoya disease: a systematic review and meta-analysis. Neurol India. 2013;61:35–9.
27. Kleinloog R, Regli L, Rinkel GJ, Klijn CJ. Regional differences in incidence and patient characteristics of moyamoya disease: a systematic review. J Neurol Neurosurg Psychiatry. 2012;83:531–6.
28. Sonobe S, Fujimura M, Niizuma K, Nishijima Y, Ito A, Shimizu H, 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.
29. Kanoke A, Fujimura M, Niizuma K, Ito A, Sakata H, Sato-Maeda M, 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.
30. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, et al. Genetics and biomarkers of moyamoya disease: significance of *RNF213* as a susceptibility gene. Journal of stroke. 2014;16:65–72.
31. Ito A, Fujimura M, Niizuma K, Kanoke A, Sakata H, Morita-Fujimura Y, et al. Enhanced postischemic angiogenesis in mice lacking *Rnf213*; a susceptibility gene for moyamoya disease. Brain Res. 2015;1594:310–20.
32. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, 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.
33. Kobayashi H, Matsuda Y, Hitomi T, Okuda H, Shioi H, Matsuda T, et al. Biochemical and functional characterization of *RNF213* (mysterin) R4810K, a susceptibility mutation of moyamoya disease, in angiogenesis in vitro and in vivo. J Am Heart Assoc. 2015;4:e002146.
34. Ohkubo K, Sakai Y, Inoue H, Akamine S, Ishizaki Y, Matsushita Y, et al. Moyamoya disease susceptibility gene *RNF213* links inflammatory and angiogenic signals in endothelial cells. Sci Rep. 2015;5:13191.
35. Kim SJ, Heo KG, Shin HY, Bang OY, Kim GM, Chung CS, et al. Association of thyroid autoantibodies with moyamoya-type cerebrovascular disease: a prospective study. Stroke. 2010;41:173–6.
36. Bower RS, Mallory GW, Nwojo M, Kudva YC, Flemming KD, Meyer FB. Moyamoya disease in a primarily white, midwestern us population: increased prevalence of autoimmune disease. Stroke. 2013;44:1997–9.
37. Li H, Zhang ZS, Dong ZN, Ma MJ, Yang WZ, Han C, et al. Increased thyroid function and elevated thyroid autoantibodies in pediatric patients with moyamoya disease: a case-control study. Stroke. 2011;42:1138–9.
38. Koizumi A, Kobayashi H, Liu W, Fujii Y, Senevirathna ST, Nanayakkara S, et al. P.R4810k, a polymorphism of *RNF213*, the susceptibility gene for moyamoya disease, is associated with blood pressure. Environ Health Prev Med. 2013;18:121–9.
39. Bang OY, Chung JW, Cha J, Lee MJ, Yeon JY, Ki CS, et al. A polymorphism in *RNF213* is a susceptibility gene for intracranial atherosclerosis. PLoS One. 2016;11:e0156607.
40. Yoshihara T, Taguchi A, Matsuyama T, Shimizu Y, Kikuchi-Taura A, Soma T, et al. Increase in circulating cd34-positive cells in patients with angiographic evidence of moyamoya-like vessels. J Cereb Blood Flow Metab. 2008;28:1086–9.
41. Kim JH, Jung JH, Phi JH, Kang HS, Kim JE, Chae 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.
42. Jung KH, Chu K, Lee ST, Park HK, Kim DH, Kim JH, et al. Circulating endothelial progenitor cells as a pathogenetic marker of moyamoya disease. J Cereb Blood Flow Metab. 2008;28:1795–803.

43. Lee JY, Moon YJ, Lee HO, Park AK, Choi SA, Wang KC, 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.
44. Kang HS, Moon YJ, Kim YY, Park WY, Park AK, Wang KC, et al. Smooth-muscle progenitor cells isolated from patients with moyamoya disease: novel experimental cell model. J Neurosurg. 2014;120:415–25.
45. Rafat N, Beck G, Pena-Tapia PG, Schmiedek P, Vajkoczy P. Increased levels of circulating endothelial progenitor cells in patients with moyamoya disease. Stroke. 2009;40:432–8.
46. Kang HS, Kim JH, Phi JH, Kim YY, Kim JE, Wang KC, et al. Plasma matrix metalloproteinases, cytokines and angiogenic factors in moyamoya disease. J Neurol Neurosurg Psychiatry. 2010;81:673–8.
47. Nanba R, Kuroda S, Ishikawa T, Houkin K, Iwasaki Y. Increased expression of hepatocyte growth factor in cerebrospinal fluid and intracranial artery in moyamoya disease. Stroke. 2004;35:2837–42.
48. Park YS, Jeon YJ, Kim HS, Chae KY, Oh SH, Han IB, et al. The role of vegf and kdr polymorphisms in moyamoya disease and collateral revascularization. PLoS One. 2012;7:e47158.
49. Kim SK, Yoo JI, Cho BK, Hong SJ, Kim YK, Moon JA, et al. Elevation of crabp-i in the cerebrospinal fluid of patients with moyamoya disease. Stroke. 2003;34:2835–41.
50. Fujimura M, Watanabe M, Narisawa A, Shimizu H, Tominaga T. Increased expression of serum matrix metalloproteinase-9 in patients with moyamoya disease. Surg Neurol. 2009; 72:476–80; discussion 480.
51. Kang HS, Kim SK, Cho BK, Kim YY, Hwang YS, Wang KC. Single nucleotide polymorphisms of tissue inhibitor of metalloproteinase genes in familial moyamoya disease. Neurosurgery 2006;58:1074–80; discussion 1074–1080.
52. He J, Wang R, Zhang D, Zhang Y, Zhang Q, Zhao J. Expression of circulating vascular endothelial growth factor-antagonizing cytokines and vascular stabilizing factors prior to and following bypass surgery in patients with moyamoya disease. Exp Ther Med. 2014;8:302–8.
53. Hoshimaru M, Takahashi JA, Kikuchi H, Nagata I, Hatanaka M. Possible roles of basic fibroblast growth factor in the pathogenesis of moyamoya disease: an immunohistochemical study. J Neurosurg. 1991;75:267–70.
54. Wang X, Zhang Z, Liu W, Xiong Y, Sun W, Huang X, et al. Impacts and interactions of *PDGFRB*, *MMP*-3, *TIMP*-2, and *RNF213* polymorphisms on the risk of moyamoya disease in han chinese human subjects. Gene. 2013;526:437–42.
55. Young AM, Karri SK, Ogilvy CS, Zhao N. Is there a role for treating inflammation in moyamoya disease?: A review of histopathology, genetics, and signaling cascades. Front Neurol. 2013;4:105.
56. Jeon JS, Ahn JH, Moon YJ, Cho WS, Son YJ, Kim SK, et al. Expression of cellular retinoic acid-binding protein-i (crabp-i) in the cerebrospinal fluid of adult onset moyamoya disease and its association with clinical presentation and postoperative haemodynamic change. J Neurol Neurosurg Psychiatry. 2014;85:726–31.
57. Fukui M, Kono S, Sueishi K, Ikezaki K. Moyamoya disease. Neuropathology. 2000;20(Suppl):S61–4.
58. Soriano SG, Cowan DB, Proctor MR, Scott RM. Levels of soluble adhesion molecules are elevated in the cerebrospinal fluid of children with moyamoya syndrome. Neurosurgery. 2002;50:544–9.
59. Frank PG, Woodman SE, Park DS, Lisanti MP. Caveolin, caveolae, and endothelial cell function. Arterioscler Thromb Vasc Biol. 2003;23:1161–8.
60. Liu J, Wang XB, Park DS, Lisanti MP. Caveolin-1 expression enhances endothelial capillary tubule formation. J Biol Chem. 2002;277:10661–8.
61. Chang SH, Feng D, Nagy JA, Sciuto TE, Dvorak AM, Dvorak HF. Vascular permeability and pathological angiogenesis in caveolin-1-null mice. Am J Pathol. 2009;175:1768–76.

62. Sonveaux P, Martinive P, DeWever J, Batova Z, Daneau G, Pelat M, et al. Caveolin-1 expression is critical for vascular endothelial growth factor-induced ischemic hindlimb collateralization and nitric oxide-mediated angiogenesis. Circ Res. 2004;95:154–61.
63. Morais C, Ebrahem Q, Anand-Apte B, Parat MO. Altered angiogenesis in caveolin-1 gene-deficient mice is restored by ablation of endothelial nitric oxide synthase. Am J Pathol. 2012;180:1702–14.
64. Sbaa E, Dewever J, Martinive P, Bouzin C, Frerart F, Balligand JL, et al. Caveolin plays a central role in endothelial progenitor cell mobilization and homing in sdf-1-driven postischemic vasculogenesis. Circ Res. 2006;98:1219–27.
65. Bang OY, Chung JW, Kim SJ, Oh MJ, Kim SY, Cho YH, et al. Caveolin-1, ring finger protein 213, and endothelial function in moyamoya disease. Int J Stroke. 2016;11:999–1008.
66. Jasmin JF, Malhotra S, Singh Dhallu M, Mercier I, Rosenbaum DM, Lisanti MP. Caveolin-1 deficiency increases cerebral ischemic injury. Circ Res. 2007;100:721–9.
67. Navarro G, Borroto-Escuela DO, Fuxe K, Franco R. Potential of caveolae in the therapy of cardiovascular and neurological diseases. Front Physiol. 2014;5:370.
68. Bartel DP. Micrornas: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
69. Zeng L, He X, Wang Y, Tang Y, Zheng C, Cai H, et al. Microrna-210 overexpression induces angiogenesis and neurogenesis in the normal adult mouse brain. Gene Ther. 2014;21:37–43.
70. Dai D, Lu Q, Huang Q, Yang P, Hong B, Xu Y, et al. Serum mirna signature in moyamoya disease. PLoS One. 2014;9:e102382.
71. Park YS, Jeon YJ, Lee BE, Kim TG, Choi JU, Kim DS, et al. Association of the mir-146 ac>g, mir-196a2c>t, and mir-499a>g polymorphisms with moyamoya disease in the korean population. Neurosci Lett. 2012;521:71–5.
72. Eberhard DA, Brown MD, VandenBerg SR. Alterations of annexin expression in pathological neuronal and glial reactions. Immunohistochemical localization of annexins i, ii (p36 and p11 subunits), iv, and vi in the human hippocampus. Am J Pathol. 1994;145:640–9.
73. Herbert SP, Odell AF, Ponnambalam S, Walker JH. The confluence-dependent interaction of cytosolic phospholipase a2-alpha with annexin a1 regulates endothelial cell prostaglandin e2 generation. J Biol Chem. 2007;282:34468–78.
74. Bang OY, Fujimura M, Kim SK. The pathophysiology of moyamoya disease: an update. J Stroke. 2016;18:12–20.

Chapter 9
Genetics of Moyamoya Beyond *RNF213*: Monogenic Moyamoya Syndromes

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Abstract Moyamoya angiopathy (MMA), in addition to its idiopathic form, so-called moyamoya disease (MMD), is also part of a broad spectrum of various diseases. In that situation, it is designated by the term moyamoya syndrome (MMS). Most MMS have a strong genetic component and include a heterogeneous group of monogenic disorders with highly variable clinical features and patterns of inheritance and penetrance. The recognition of known monogenic MMS entities and the delineation of novel ones are of major importance for disease prognosis, clinical care, accurate genetic counseling, gene identification, and MMA pathomechanisms understanding. In this chapter, we will focus on (i) RASopathies, a well-known group of Mendelian diseases whose gene identification established the role of the RAS/MAPK pathway in MMA, despite the low prevalence of MMA in those

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conditions and on (ii) some recently identified very rare Mendelian conditions with a highly penetrant MMA. We will also emphasize the importance of novel high-throughput methodologies to identify the genes involved in the numerous yet uncharacterized MMS.

Keywords Moyamoya syndrome • Moyamoya disease • RASopathies • NO pathway • *BRCC3*

9.1 Introduction

The moyamoya angiopathy (MMA) was first defined as an idiopathic bilateral occlusion of distal internal carotids and/or its main branches and the development of basal collaterals [1, 2]. This idiopathic disease, designated by the term moyamoya disease (MMD), has been strongly associated with the *RNF213* p.R4810K variant in East Asian populations in which the prevalence of MMD is ten times higher than in Caucasians [3, 4]. However, it is increasingly obvious that the MMA is also part of a broad spectrum of various diseases. In that situation, it has been designated by various terms such as moyamoya syndrome (MMS) or quasi-moyamoya disease. A nationwide survey conducted in Japan in 2006 showed that the prevalence and annual incidence of MMD were 5/100,000 and 1.13/100,000, whereas those of MMS/quasi-MMD were 0.34/100,000 and 0.11/100,000 [5]. The proportion of MMS (including monogenic MMS) versus MMD seems to be higher in Western countries than in East Asian countries [6, 7]. However, rigorous epidemiological studies of moyamoya in Europe and the USA would be needed to establish this MMS/MMD ratio in Caucasian patients. The difference may relate partly to the epidemiology of underlying conditions. For example, sickle-cell disease is a frequent cause of moyamoya in people from African origin, who are more numerous in the USA and in Europe than in Asia. Interestingly, MMA prevalence in Afro-Americans and white Caucasians is similar when patients with sickle-cell disease are excluded [8].

Ten to fifteen percent of MMD cases are familial. The pattern of inheritance in familial MMD cases is so far unclear and most likely heterogeneous, based on the observed familial aggregation. The number of affected members in MMD families with more than one case is most often limited, suggesting an oligogenic or multifactorial inheritance. However, a few large MMD families consistent with an autosomal dominant pattern of inheritance have been reported in Japan [9]. In those families, affected cases share the *RNF213* p.R4810K [4].

Most MMS (except radiation-induced MMS) have a strong genetic component. Diseases associated with MMA include a number of either polygenic/multifactorial conditions (particularly autoimmune disorders), chromosomal anomalies, or monogenic, so-called Mendelian, disorders [10]. Monogenic disorders associated with MMA constitute a highly heterogeneous group with either autosomal dominant, autosomal recessive, or X-linked pattern of inheritance. The penetrance of MMA is also highly variable. Indeed, the proportions of affected cases showing MMA range

from less than 2% in most of these conditions, such as Noonan syndrome (NS), to 90% in *BRCC3*-mutated patients [10]. Several MMS causative genes have been identified including genes involved in the RAS, genomic maintenance, or nitric oxide (NO) pathways. However, in most MMS, the genes have yet to be identified.

The recognition of known monogenic MMS entities and the delineation of novel ones are of major importance for disease prognosis, clinical care, accurate genetic counseling, gene identification, and MMA pathomechanisms understanding. Indeed, identification of MMS genes whose mutations would lead to a highly penetrant MMA could provide important clues to the cellular pathways involved in the pathogenesis of this highly heterogeneous condition and to the "moyamoya interactome."

9.2 Heterogeneity of Monogenic Moyamoya Syndromes

MMA has been reported in several Mendelian diseases whose causative genes have been identified [10]. These MMS constitute a highly heterogeneous group regarding prevalence, clinical presentation, and patterns of inheritance and penetrance of the MMA. Monogenic diseases with a minimum of two reported unrelated patients with MMA are presented in the enclosed Table 9.1.

In the following sections, we choose to focus on (i) RASopathies, a well-known group of Mendelian diseases whose gene identification established the role of the RAS/MAPK pathway in MMA, despite the low prevalence of MMA in those conditions and on (ii) some recently identified very rare Mendelian conditions with a highly penetrant MMA.

9.2.1 Monogenic Moyamoya Syndromes Caused by Mutations in the RAS/MAPK Pathway Genes

RASopathies are a group of phenotypically overlapping conditions caused by germline mutations of genes encoding components of the RAS/MAPK signaling pathway. [11] The vast majority of these mutations leads to an increased signal transduction down the RAS/MAPK pathway. Several Mendelian autosomal dominant RASopathies have been associated with MMA, including neurofibromatosis type 1 (NF1), Noonan syndrome (NS), and Noonan-like syndromes. Despite the low penetrance of MMA in these conditions, its association with RASopathies has long been known since the prevalence of NF1 and NS is close to 1 out of 3000, among the highest prevalences of Mendelian diseases.

Table 9.1 Monogenic moyamoya syndromes

Disease name, pattern of inheritance, and mutated gene(s)	Disease prevalence	Proportion or number of MMA reported cases	Hallmark symptoms of the disease
Neurofibromatosis type 1	1/3000	2–7% of NF1 cases have a cerebral vasculopathy	"Café au lait" macules
NF1			Cutaneous/subcutaneous neurofibroma
AD		50% of NF1 patients with a cerebral vasculopathy have MMA	Axillary or groin freckling
			Optic pathway glioma
LOF mutations leading to an activation of the RAS pathway			Lisch nodules (iris hamartomas)
			Bone dysplasia
		>70 MMA reported cases	Additional malignant tumors
			Possible systemic vasculopathy (aortic coarctation, renal artery stenosis…)
Noonan syndrome	1/2500	9 cases	Short stature
PTPN11 (50% NS patients)			Congenital heart defect (pulmonary valve stenosis, hypertrophic cardiomyopathy…)
SOS1			
RAF1			Developmental delay
KRAS, *NRAS*, *BRAF*, *MAP2K1*			Dysmorphism (broad or webbed neck, unusual chest shape, low-set nipple, facial dysmorphism)
AD			
GOF mutations leading to an activation of the RAS pathway			Cryptorchidism, male infertility
			Ocular abnormalities
			Cutaneous abnormalities ("café au lait" macules, lentigines)

(continued)

Table 9.1 (continued)

Disease name, pattern of inheritance, and mutated gene(s)	Disease prevalence	Proportion or number of MMA reported cases	Hallmark symptoms of the disease
Alagille syndrome	1/70,000	1/268 in one study	Quasi-constant symptoms (>80%):
JAG1 in most cases (60% de novo mutations)			Cholestasis
NOTCH2 in less than 1% of cases			Congenital heart disease: pulmonary artery stenosis > others
			Butterfly vertebrae
AD			Posterior embryotoxon
			Less frequent symptoms:
			Dysmorphic face
			Systemic angiopathy (aortic coarctation or aneurysms, renal artery stenosis…)
			Renal anomalies
			Growth retardation
			Pancreatic insufficiency
			Learning difficulties
Sickle-cell disease	Variable according to ethnic origins	Moyamoya collateral vessels in 20–40% of patients with sickle-cell disease who have a history of stroke	Vaso-occlusive events (bone and organs) leading to acute or chronic pain and organ dysfunction
HBB			Chronic hemolytic anemia
AR	Higher prevalence in African, Afro-American, Caribbean, or Mediterranean populations		Increased risk of bacterial infections
MMA with achalasia	?	5 families reported	Severe achalasia
GUCY1A3		MMA in 1/3 of subjects	Hypertension
			Raynaud syndrome, livedo
AR			Erectile dysfunction
LOF mutations in the α1-chain of the major NO receptor, leading to an alteration of the NO pathway			Low platelet count

(continued)

Table 9.1 (continued)

Disease name, pattern of inheritance, and mutated gene(s)	Disease prevalence	Proportion or number of MMA reported cases	Hallmark symptoms of the disease
MMA with *SAMHD1* mutations	?	3 families reported	Aicardi-Goutieres syndrome
SAMHD1		MMA in about 50% of mutation carriers	Aseptic subacute (relapsing) encephalopathy in early childhood leading to psychomotor regression and epilepsy
AR			
LOF mutations			Chilblains, poor adaptation to cold
			MRI: leukodystrophy, cerebral calcifications
			CSF: lymphocytic meningitis, high interferon-alpha and neopterin levels
			Other symptoms in *SAMHD1* mutations:
			Congenital glaucoma
			Arthritis
MOPDII or Majewski syndrome (microcephalic primordial dwarfism)	?	More than 50 cases reported	Intrauterine growth retardation
			Severe proportionate short stature
PCNT		Cerebral arteriopathy in 19–52% of subjects	Microcephaly
			Bone radiologic abnormalities
		MMA in most PCNT patients with a cerebral arteriopathy	Absent or mild mental retardation
LOF mutations in the pericentrin protein leading to various functional consequences including alteration of genomic maintenance			Insulin resistance

(continued)

Table 9.1 (continued)

Disease name, pattern of inheritance, and mutated gene(s)	Disease prevalence	Proportion or number of MMA reported cases	Hallmark symptoms of the disease
Seckel syndrome (microcephalic primordial dwarfism)	1/10,000	2 case reports	Intrauterine growth retardation
			Severe proportionate short stature
			Microcephaly
ATR			Characteristic "bird-headed" facial appearance
RBBP8			
CENPJ			Absence of skeletal dysplasia
CEP152			Mental retardation
CEP63			
NIN			
AR			
LOF in genes involved in cycle cell progression, centrosomal function, or DNA repair			
MMA with hypergonadotropic hypogonadism, dysmorphism, and cardiopathy	?	4 families reported including one with an F8 hemophilia	Constant or frequent (>50%) symptoms:
			Short stature
X-linked recessive		9/10 patients had MMA	Facial dysmorphism (hypertelorism, long philtrum, mild ptosis)
Xq28 deletion leading to loss of expression of ***BRCC3 and MTCP1***			Hypergonadotropic hypogonadism
			Premature hair graying
Loss-of-function mutations			Less frequent symptoms (<50%):
			Premature coronary heart disease
			Arterial hypertension
			Early-onset cataract
			Developmental delay

(continued)

Table 9.1 (continued)

Disease name, pattern of inheritance, and mutated gene(s)	Disease prevalence	Proportion or number of MMA reported cases	Hallmark symptoms of the disease
Pseudo moyamoya with ACTA 2 mutations	?	Quasi-constant cerebral angiopathy in ACTA2 R179 mutations	Constant features:
			TAAD
ACTA2			Premature coronary heart disease
AD			
Unknown functional consequences			Livedo reticularis
		No basal collateral network	Features specific to R179H mutations:
			Persistent ductus arteriosus (constant)
			Congenital mydriasis (constant)
			Arterial pulmonary hypertension
			Hypotonic bladder
			Malrotation and hypoperistalsis of the gut

Monogenic disorders including at least two published patients with a well-defined moyamoya angiopathy. *Bold* genes: genes shown to be mutated in monogenic MMS patients, *GOF* gain of function, *ICA* internal carotid artery, *LOF* loss of function, *MMA* moyamoya angiopathy, *MMS* moyamoya syndrome, *TAAD* thoracic aortic aneurysms and dissections, and *sGC* soluble guanylate cyclase

9.2.1.1 Neurofibromatosis Type 1

NF1 is an autosomal dominant disorder affecting approximately 1 in 3000 newborns which is caused by heterozygous loss-of-function mutations in the *NF1* gene [12]. The *NF1* gene is a tumor suppressor gene which encodes neurofibromin, a GTPase-activating protein that negatively regulates Ras. Close to 50% of patients are sporadic cases harboring de novo mutations. It is a pleiotropic disorder associating café au lait maculae, cutaneous freckling, neurofibromas, iris hamartomas, and a predisposition to various malignant tumors. In addition, brain and periphery vasculopathies are important features of this condition.

More than 70 pediatric NF1 cases with a cerebral vasculopathy have been reported so far in the literature [13–16]. Between 2 and 7% of NF1 pediatric cases who undergo cerebral neuroimaging have a cerebral vasculopathy, which is in half cases an MMA. MMA features in *NF1*-mutated patients are very similar to those observed in MMD patients [17]. These data strongly suggest that *NF1* is the RASopathy with the highest association with MMA, 1–4% of NF1 cases showing

an MMA. However, additional environmental and genetic factors most likely play a role in the occurrence of MMA in NF1 patients. Indeed, it has been strongly established that *NF1* is the main genetic factor of radiation-induced cerebral vasculopathy [18]. In addition, the presence of the pR4810K variant has been strongly associated with MMA in a cohort of NF1 Korean patients [19].

The mechanisms of cerebral vasculopathy and MMA, which seem acquired and not congenital abnormalities in NF1 patients, are unknown. Neurofibromin is expressed in endothelial cells (EC) and vascular smooth muscle cells (VSMC). NF1 vascular lesions are characterized by an intima hyperplasia leading to stenosis and it has been hypothesized that loss of *NF1* in cells of the vascular wall might lead to altered vascular maintenance and repair processes [20]. Indeed, NF1+/− mice have been shown to have increased neointima formation in response to mechanical stress, increased numbers of proliferative VSMC, and MAPK/ERK activation [21]. The cellular components involved in this process might include not only EC and VSMC but also bone marrow-derived cells [22]. Altogether, these data provide a framework for understanding the pathogenesis of NF1 vasculopathy, including MMA.

9.2.1.2 Noonan Syndrome

NS is a relatively common, pleiotropic, developmental disorder affecting approximately 1/2500 newborns [23]. It is characterized by variously associated features including reduced postnatal growth, craniofacial dysmorphism, cardiovascular defects (most often pulmonary stenosis), variable cognitive deficits, bleeding tendency, skeletal defects, cryptorchidism, lymphangiectasia, and an increased risk to childhood hematologic malignancies, including juvenile myelomonocytic leukemia (JMML). Diagnosis is most often made by clinical examination. However, the diagnosis can be difficult in mild forms or in the adult since the phenotype is less pronounced with age. Four genes, *PTPN11*, *KRAS*, *SOS1*, and *RAF1*, account for 70% of NS patients. *PTPN11* which encodes the tyrosine phosphatase SHP2 protein accounts for 50% of NS.

The occurrence of MMA in an NS patient was first reported in 1997 [24]. Since then, eight other cases have been reported to our knowledge [25–28]. Although the proportion of NS patients showing MMA has not been investigated in large series of patients, the limited number of reported cases strongly suggests that the penetrance of MMA in NS is quite low. This very limited number of NS patients with MMA and the absence of molecular data in most of them preclude any genotype-phenotype correlation. Clinical and molecular characterization of larger number of NS patients with MMA would be of importance to better define clinical management [28]. The mechanisms leading from NS gene mutations to MMA are so far unknown, but the association of this rare cerebrovascular angiopathy with NS in several patients strongly suggests that a dysregulation of the RAS/MAPK pathway is involved.

9.2.1.3 Noonan-Like Syndromes

Two distinct NS-like syndromes caused by *SHOC2* and *CBL* gene mutations have been associated with moyamoya. The NS-like syndrome with loose anagen hair, which is caused by a *SHOC2* mutation, has been initially described in 2003 [29]. This autosomal dominant syndrome associates some of the NS features with several additional features, including macrocephaly, proven growth hormone (GH) deficiency, distinctive hyperactive behavior, increased skin pigmentation, and hair anomalies called loose anagen hair. This syndrome is caused by a unique Ser2Gly mutation in the *SHOC2* gene which introduces an N-myristoylation site in this positive modulator of the Ras signaling pathway, leading to its constitutive membrane translocation [30]. Three children with moyamoya and a *SHOC2* p.Ser2Gly mutation have recently been reported in Korea and Taiwan [31, 32]. The clinical features of these three cases, besides MMA, were very similar to those previously reported in *SHOC2*-mutated patients.

Germline missense heterozygous mutations in *CBL* have been shown to cause a developmental disorder sharing some of the clinical features (impaired growth, developmental delay, cryptorchidism, increased risk for JMML) encountered in NS patients, although facial dysmorphic features of these patients may be subtle [33–35]. The *CBL* gene encodes a RING finger E3 ubiquitin ligase that acts as a negative regulator of Ras signaling. CBL protein contains a tyrosine kinase-binding domain and a RING finger domain that mediates the E3 ubiquitin ligase activity. These two domains are separated by a linker sequence which is crucial for the ubiquitin ligase activity of the protein. It controls proliferative signals via the ubiquitination of activated growth factor receptor tyrosine kinases, endocytosis, and degradation of these receptors. In addition, CBL is a multiadaptor protein that controls intracellular trafficking.

Heterozygous germline *CBL* mutations are encountered in a small portion of patients (less than 1%) whose features fit NS [34]. Among those, two patients were very recently reported to have moyamoya [36, 37]. The first one was a 7-year-old girl, previously diagnosed at 1 y.o. with JMML[37], who carried a highly recurrent JMML mutation, p.Tyr371His. The second one, initially referred for splenomegaly and xanthogranuloma, was diagnosed as having moyamoya at age 14 y.o. [36]. She carried a p.Arg420Gln mutation located in the *CBL* ring finger domain. It would be important in the next future to investigate the putative role of *CBL* mutations in the development of MMA in non-JMML patients.

9.2.2 *Recently Identified Highly Penetrant Monogenic Moyamoya Syndromes*

9.2.2.1 X-Linked MMS and BRCC3/MTCP1 Deletion

A recessive X-linked MMS due to loss of expression of *BRCC3/MTCP1* was recently reported in three families [38, 39]. All patients were males, and pedigrees showed a maternal inheritance. Bilateral moyamoya angiopathy was present in nine out of ten mutation carriers, a penetrance which is very high and much higher than in any other MMS ever reported. Age at onset of acute neurological symptoms ranged from 4 to 32 years. Other frequent symptoms include short stature of postnatal onset, hypergonadotropic hypogonadism, stereotyped facial dysmorphism, and heart involvement (isolated left ventricular enlargement or symptomatic dilated cardiomyopathy). Inconstantly, patients presented arterial hypertension, premature coronary heart disease, and early-onset bilateral cataract. Developmental delay was reported in one family. This stereotyped phenotype has been observed in four additional families (FR & ETL personal communication). The disease is caused by an Xq28 deletion that leads to a complete loss of expression of *BRCC3 and MTCP1* genes. Interestingly, patients showing a similar phenotype associated with hemophilia have been reported to carry an Xq28 deletion encompassing *BRCC3* and *F8* [40].

BRCC3 (also called BRCC36) is a ubiquitously expressed K63-specific deubiquitinating (DUB) enzyme containing a JAMM (JAB1/MPN/Mov34 metalloenzyme) domain [41]. It is a member of two distinct protein complexes, the nuclear BRCA1 DNA repair complex and the cytoplasmic BRISC isopeptidase complex [42]. The BRCA1 complex plays a major role in DNA double-strand breaks repair pathway. The biological function of the BRISC complex is still largely unknown, but recent data unraveled its role in two cellular pathways. Firstly, the BRISC complex has been involved in spindle mitotic assembly [43]. The BRISC complex has also been shown to deubiquitinate IFNAR1, one of the two chains of the interferon-alpha (IFN) receptor, thus limiting its endocytosis and controlling cellular responses to IFN [44]. BRISC-deficient cells and mice exhibit attenuate responses to IFN and are protected from IFN-associated immunopathology. How alterations of anyone of these pathways could lead to MMA would be speculative at this point. However, several lines of evidence suggest possible links between MMA and genomic maintenance, such as the role of neck irradiation as a major environmental factor for MMA and the association of MMA with pericentrin and Seckel primordial dwarfisms [45]. Interestingly, *RNF213* has also been involved in genomic maintenance [46]. As regards the IFN pathway, mutations of *SAMHD1*, a gene encoding a triphosphohydrolase enzyme that controls the intracellular level of dNTPs and the innate immune response, have also been associated with MMA in several families [47]. The development of conditional *BRCC3* mouse mutants leading to the ablation of *BRCC3* in various cellular compartments including EC, VSMC, and bone marrow-derived cells would be an important tool to sort out the pathomechanisms of this highly penetrant MMS.

9.2.2.2 MMS Caused by Mutations of GUCY1A3, Encoding the α1-Chain of the Major Nitric Oxide Receptor

Another MMS associating moyamoya and achalasia was identified recently [48]. It is caused by mutations in *GUCY1A3*, which encodes the α1-subunit of soluble guanylate cyclase (sGC), the major receptor for nitric oxide (NO). This receptor is a heterodimer composed of one α-chain and a β-chain [49]. Three consanguineous families including a total of nine affected children (six males and three females) were reported. Early-onset moyamoya angiopathy led to ischemic events occurring before 2 years of age. Moyamoya was unilateral or bilateral, and the posterior circulation could be affected. Three cases with intracranial angiopathy had typical MMA angiopathy. One additional child presented a unilateral long arterial stenosis of MCA and ACA without moyamoya neovessels network. All mutation carriers also had a severe achalasia, leading to regurgitations during the first weeks of life. Other hallmark features were arterial hypertension, present in 50% of affected subjects, and more rarely, vasomotor dysfunction such as Raynaud phenomenon or livedo, erectile dysfunction, and low platelet count without history of abnormal bleeding. In each family, a homozygous loss-of-function mutation was identified in affected subjects that led to absence of expression of the protein (one nonsense mutation, one splice-site mutation, and one frameshift deletion). Two additional unrelated *GUCY1A3* mutation carriers have recently been reported [50]. The phenotype of these two patients was similar to the one observed in the three families previously reported with the exception of the absence of achalasia in one of these patients, aged 3 y.o., and the late onset of achalasia in the second one. Interestingly, the patient without achalasia was a compound heterozygote with a frameshift and a missense mutation.

GUCY1A3 encodes the most abundant α-isoform of sGC, an NO-dependent enzyme that regulates SMC relaxation in the vascular and extravascular systems. A striking feature of this condition is the apparent cerebrovascular preference of vascular lesions, the sole other vascular symptoms encountered in some of these patients being hypertension and livedo. The mechanisms underlying this cerebrovascular selectivity are so far unknown. The histological analysis of internal carotid arteries (ICA) in constitutive C57Bl6 mouse mutants ablated for *GUCY1A3* did not show stenosis of ICA bifurcations (Boulday G & ETL personal communication). Sensitization of these mouse models or the use of more severe sGC mouse mutants, such as sGC β-chain-ablated mouse mutants, might provide relevant MMA mouse models.

9.3 Clinical Implications and Future Challenges in Monogenic Moyamoya Syndromes

Monogenic MMS constitute a very heterogeneous group with various clinical presentations and patterns of inheritance. The penetrance of the MMA is also highly variable, from less than 2% in most RASopathies to more than 90% in *BRCC3*-associated MMS. The diagnostic of a known monogenic MMS entity in a given patient through its specific clinical features and/or its molecular signature is of major importance for disease prognosis, clinical care, and accurate genetic counseling. For example, the identification of a *BRCC3* deletion in a male child with an MMA should lead to a tight cardiac follow-up, and his female relatives should be provided adequate genetic counseling. Another example relates to the hematological follow-up to be proposed to MMS patients with a *CBL* mutation. Molecular diagnostic of these various MMS could be oriented by specific associated clinical features. For example, the association of MMA with achalasia should lead to a *GUCY1A3* screening, whereas the presence of Noonan features should lead to screening of NS genes. However, in some patients, specific features are mild or absent, raising the question of the sequencing of a targeted panel of MMS genes using currently available high-throughput sequencing methodologies.

In addition to clinical care improvement, the identification of the genes involved in several monogenic MMS unraveled different pathways involved in the development of this angiopathy, for example, the NO, RAS, and NOTCH pathways as well as pathways involved in genomic maintenance. Some of these genes, such as *BRCC3*, are involved in more than one pathway, and it is not yet clear which one(s) are involved in MMA pathogenesis. The development of MMA animal models through the conditional ablation of one of the recently identified highly penetrant MMS conditions would be of major importance for a better understanding of MMA mechanisms and the identification of druggable therapeutical targets.

Altogether these data suggest that various signaling pathways may be involved in MMA determination and progression. These pathways might cross each other at some point. However, most genes causing MMA in MMS patients are yet unknown, and the putative functional links between the candidate pathways already identified are unclear. Identification of most monogenic MMS genes could provide important clues to the cellular pathways involved in the pathogenesis of this highly heterogeneous condition and the "moyamoya interactome." Recent technological developments in genomics now allow the entire coding regions (exome) to be interrogated for rare genetic variants, and candidate genes for Mendelian disorders can now be identified by whole-exome sequencing even in small families [51]. Identification of those so far unknown MMS genes will be the basis for future projects aiming at the development of cellular models and an animal model, both urgently needed for this disease.

References

1. Scott RM, Smith ER. Moyamoya disease and moyamoya syndrome. N Engl J Med. 2009;360:1226–37.
2. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. Lancet Neurol. 2008;7:1055–66.
3. 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(1):34–40.
4. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542.
5. Hayashi K, Horie N, Suyama K, Nagata I. An epidemiological survey of moyamoya disease, unilateral moyamoya disease and quasi-moyamoya disease in Japan. Clin Neurol Neurosurg. 2013;115(7):930–3.
6. Khan N, Schuknecht B, Boltshauser E, et al. Moyamoya disease and Moyamoya syndrome: experience in Europe; choice of revascularisation procedures. Acta Neurochir. 2003;145(12):1061–71.
7. Kraemer M, Heienbrok W, Berlit P. Moyamoya disease in Europeans. Stroke. 2008;39(12):3193–200.
8. Uchino K, Johnston SC, Becker KJ, Tirscwell DL. Moyamoya disease in Washington state and California. Neurology. 2005;65:956–8.
9. Mineharu Y, Liu W, Inoue K, et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70:2357–63.
10. Guey S, Tournier-Lasserve E, Herve D, Kossorotoff M. Moyamoya disease and syndromes: from genetics to clinical management. Appl Clin Genet. 2015;8:49–68.
11. Tidyman WE, Rauen KA. The RASopathies: developmental syndrome of RAS/MAPK pathway dysregulation. Curr Opin Genet Dev. 2009;19:230–6.
12. Hirbe AC, Gutmann DH. Neurofibromatosis type 1: a multidisciplinary approach to care. Lancet Neurol. 2014;13:834–43.
13. Cairns AG, North KN. Cerebrovascular dysplasia in neurofibromatosis type 1. J Neurol Neurosurg Psychiatry. 2008;79:1165–70.
14. Rosser TL, Vezina G, Packer RJ. Cerebrovascular abnormalities in a population of children with neurofibromatosis type 1. Neurology. 2005;64:553–5.
15. Sobata E, Ohkuma H, Suzuki S. Cerebrovascular disorders associated with von Recklinghausen's neurofibromatosis: a case report. Neurosurgery. 1988;22:542–9.
16. Kaas B, Huisman TA, Tekes A, Bergner A, Blakeley JO, Jordan LC. Spectrum and prevalence of vasculopathy in pediatric neurofibromatosis type. J Child Neurol. 2013;28:561–9.
17. Koss MK, Scott M, Irons MB, Smith ER, Ullrich NJ. Moyamoya syndrome associated with neurofibromatosis type 1: perioperative and long-term outcome after surgical revascularization. J Neurosurg Pediatrics. 2013;11:417–25.
18. Grill J, Couanet D, Cappelli C, Habrand JL, Rodriguez D, Sainte-Rose C, Kalifa C. Radiation-induced cerebral vasculopathy in children with neurofibromatosis and optic pathway glioma. Ann Neurol. 1999;45:393–6.
19. Phi JH, Choi JW, Seong MW, Kim T, Moon YJ, Lee J, et al. Association between Moyamoya syndrome and the *RNF213* c.14576G>A variant in patients with neurofibromatosis type 1. J Neurosurg Pediatr. 2016 Feb;5:1–6.
20. Hamilton SJ, Friedman JM. Insights into the pathogenesis of neurofibromatosis 1 vasculopathy. Clin Genet. 2000;58:341–4.
21. Xu J, Ismat FA, Wang T, Yang J, Epstein JA. NF1 regulates a Ras-dependent vascular smooth muscle proliferative injury response. Circulation. 2007;116:2146–58.
22. Lasater EA, Li F, Bessler WK, Estes ML, Vemula S, Hingtgen CM, Dinauer MC, et al. Genetic and cellular evidence of vascular inflammation in neurofibromin-deficient mice and humans. J Clin Invest. 2010;120:859–70.

23. Tartaglia M, Zampino G, Gelb BD. Noonan syndrome: clinical aspects and molecular pathogenesis. Mol Syndromol. 2010;1:2–16.
24. Ganesan V, Kirkham FJ. Noonan syndrome and moyamoya. Pediatr Neurol apr. 1997;16(3):256–8.
25. Schuster JM, Roberts TS. Symptomatic Moyamoya disease and aortic coarctation in a patient with Noonan's syndrome. Pediatr Neurosurg. 1999;30:206–10.
26. Tang KT, Yang W, Wong J, Lee KY. Noonan syndrome associated with Moyamoya disease: report of one case. Acta Paediatr Taiwan. 1999;40:274–6.
27. Yamashita Y, Kusaga A, Koga Y, Nagamitsu S, Matsuishi T. Noonan syndrome moyamoya-like vascular changes and antiphospholipid syndrome. Pediatr Neurol. 2004;31:364–6.
28. Gupta M, Choudhri OA, Feroze AH, Do HM, Gant GA, Steinberg GK. Management of moyamoya syndrome in patients with Noonan syndrome. J Clin Neurosci. 2016;28:107–11.
29. Mazzanti L, Cacciari E, Cicognani A, Bergamaschi R, Scarano E, Forabosco A. Noonan-like syndrome with loose anagen hair: a new syndrome. Am J Med Genet A. 2003;118A(3):279–86.
30. Cordeddu V, Di Schiavi E, Pennachio LA, Ma'ayan A, Sarkozy A, Fodale V, et al. Mutation in *SHOC2* promotes aberrant protein N-myristoylation and underlies Noonan-like syndrome with loose anagen hair. Nat Genet. 2009;41:1022–6.
31. Choi JH, Oh MY, Yum MS, Lee BH, Kim GH, Yoo HW. Moyamoya syndrome in a patient with Noonan-like syndrome with loose anagen hair. Pediatr Neurol. 2015;52:352–5.
32. Lo FS, Wang CJ, Wong MC, Lee NC. Moyamoya disease in 2 patients with Noonan-like syndrome with loose anagen hair. Am J Med Genet Part A. 2015;167:1285.
33. Niemeyer CM, Kang MW, Shin DH, Furtan I, Erlacher M, Bunin NJ, et al. Germline *CBL* mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010;42:794–800.
34. Martinelli S, De Luca A, Stellacci E, Rossi C, Checquolo S, Lepri F, et al. Heterozygous germline mutations in the *CBL* tumor suppressor gene cause a noonan syndrome-like phenotype. Am J Hum Genet. 2010;87:250–7.
35. Perez B, Mechinaud F, Galambrun C, Ben Romdhane N, Isidor B, Philip N, et al. Germline mutations of the *CBL* gene define a new genetic syndrome with predisposition to JMLL. J Med Genet. 2010;47:686–91.
36. Strullu M, Caya A, Cassinat B, Fenneteau O, Touzot F, Blauwblomme T, et al. In hematopoietic cells with a germline mutation of *CBL*, loss of heterozygosity is not a signature of juvenile myelo-monocytic leukemia. Leukemia. 2013;27:2404–7.
37. Yakuna M, Muramatsu H, Higa T, Chinen Y, Wang X, Kojima S. Germline mutation of *CBL* is associated with Moyamoya disease in a child with juvenile myelomonocytic leukemia and Noonan-like disorder. Pediatr Blood Cancer. 2015;62:542–4.
38. Hervé D, Touraine P, Verloes A, Miskinyte S, Krivosic V, Logeart D, et al. A hereditary moyamoya syndrome with multisystemic manifestations. Neurology. 2010;75:259–64.
39. Miskinyte S, Butler MG, Hervé D, et al. Loss of *BRCC3* deubiquitinating enzyme leads to abnormal angiogenesis and is associated with syndromic moyamoya. Am J Hum Genet. 2011;88(6):718–28.
40. Janczar S, Fogtman A, Koblowska M, Baranska D, Pastorczak A, Wegner O, et al. Novel severe hemophilia A and moyamoya (SHAM) syndrome caused by Xq28 deletions encompassing *F8* and *BRCC3* genes. Blood. 2014;123:4002–3.
41. Sowa ME, Bennett EJ, Gygi SP, Harper JW. Defining the human deubiquitinating enzyme interaction landscape. Cell. 2009;138:389–403.
42. Feng L, Wang J, Chen J. The Lys63-specific deubiquitinating enzyme BRCC36 is regulated by two scaffold proteins localizing in different subcellular compartments. J Biol Chem. 2010;285:30982–8.
43. Yan K, Li L, Wang X, Hong R, Zhang Y, Yang H, Lin M, et al. The deubiquitinating enzyme complex BRISC is required for proper mitotic spindle assembly in mammalian cells. J Cell Biol. 2015;210:209–14.

44. Zheng H, Gupta V, Patterson-Fortin J, Bhattacharya S, Katlinski K, Wu J. A BRISC-SHMT complex deubiquitinates IFNAR1 and regulates interferon responses. Cell Rep. 2013;5(1):180–93.
45. Delaval B, Doxsey SJ. Pericentrin in cellular function and disease. J Cell Biol. 2010;188:181–90.
46. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, et al. The moyamoya disease susceptibility variant *RNF213* R4810K (rs112735431) induces genomic instability by mitotic abnormality. Biochem Biophys Res Commun. 2013;439:419–26.
47. Rodero MP, Crow YJ. Type I interferon-mediated monogenic autoinflammation : the type 1 interferonopathies, a conceptual overview. J Exp Med. 2016;7:1–12.
48. Hervé D, Philippi A, Belbouab R, et al. Loss of α1β1 soluble guanylate cyclase, the major nitric oxide receptor, leads to moyamoya and achalasia. Am J Hum Genet. 2014;94(3):385–94.
49. Bryan NS, Bian K, Murad F. Discovery of the nitric oxide pathway and targets for drug development. Front Biosci. 2009;14:1–18.
50. Wallace S, Guo DC, Regalado E, Mellor-Crumley L, Banshad M, Nickerson D, et al. Disrupted nitric oxide signaling due to *GUCY1A3* mutations increases risk for moyamoya disease, achalasia and hypertension. Clin Genet. 2016;90:351–60.
51. Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, et al. Targeted capture and massively parallel sequencing of 12 human exomes. Nature. 2009;461:272–6.

Part IV
Clinical Management

Chapter 10
Natural History and Long-Term Clinical Outcome After Conservative and Surgical Management

Won-Sang Cho and Jeong Eun Kim

Abstract Moyamoya disease (MMD) is a rare but interesting subject in the field of cerebrovascular diseases because of its unique angiographic features and clinical characteristics, partly satisfactory surgical outcomes, and still unknown cause and remaining controversies. Here, the authors reviewed the previous studies about both pediatric and adult MMD with our experience in the management of adult patients. Taking a look at the natural history of MMD, the significance of surgical treatment for ischemic and hemorrhagic presentation was considered. Bypass surgery seems to be effective in prevention of ischemic stroke; however, the perioperative complications are the big obstacle to change the natural clinical course. Among surgical skills, combined bypass surgery is expected to have a better role than indirect surgery in the long term, in terms of the extent of flow augmentation and detailed neurological/cognitive functions. Until now, however, gross clinical outcomes look not so different between combined and indirect procedures. The effectiveness of surgery to prevent from rebleeding in hemorrhage MMD is still inconclusive. Antiplatelet medication may be effective in some situations with no increase in hemorrhagic complication. Now, randomized prospective study is needed to make a standard treatment guideline, and basic research should be kept parallel to clinical studies to find out the causes, stop the progression, and apply the appropriate treatment modalities.

Keywords Moyamoya disease • Natural history • Bypass surgery • Outcome • Complications

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10.1 Natural History of Moyamoya Disease (MMD)

10.1.1 Ischemic MMD

There have been few studies about the natural history of pure pediatric MMD [11, 19], but most of studies have dealt with mainly adult MMD with a small number of pediatric patients [6, 7, 13, 14, 20, 36, 38, 44, 56]. Choi et al. reported that 33.3% of adult and 60% of pediatric patients were aggravated in ischemic symptoms during conservative management [11]. Imaizumi et al. reported that 60% of nonsurgical patients ranged from disabled to death during follow-up [19]. Patients younger than 3–5 years tended to show rapid progression and poor surgical outcomes [32]. Although recuperative power is greater in pediatrics than adults, development delay, cognitive dysfunction, and chronic seizure as well as neurological deficits can occur in the developing brain. In such reasons, most of symptomatic pediatric patients have undergone bypass surgery, and the results are satisfactory [11, 19, 31, 40, 48].

Reviewing severe studies about the natural clinical course of adult MMD [6, 7, 13, 14, 36, 44, 55], recurrence rate of stroke such as infarction and hemorrhage is quite considerable than expected although there is some difference among ethnic groups. Here we are to think about the ischemic MMD, dealing with the hemorrhagic MMD in the latter part. According to the Hallemeier et al.'s study following 34 adult patients over 5.1 years [14], 5-year stroke occurrence was about 65%. Gross et al. reported that infarction and hemorrhage respectively occurred in 13.3%/person-year and 1.7%/person-year during 2.9-year follow-up of 42 adult patients [13]. In Chiu et al.'s study [6], 35 patients experienced 10.3%/person-year of stroke during 40 months, which consisted of 1.7% of hemorrhage and 8.6% of ischemic symptoms. In our natural course study following 241 adult MMD patients with hemodynamic stability (more than 50% of reserve capacity) and mild symptoms [7], overall stroke rate was 4.5%/person-year in which hemorrhage and ischemia were 2.3% and 2.2%, respectively. Clinical outcome was worst in patients with hemorrhagic presentation other than ischemic and asymptomatic presentations. Initial clinical presentation tended to recur, and patients with family history of MMD showed high occurrence of stroke. Noh et al.'s recent study showed that 1- and 5-year recurrence rates in patients with ischemic MMD under conservative management were 1.6% and 11.8%, respectively [46]. Risk factors of recurrent ischemic stroke included hypertension, diabetes mellitus, decrease in reserve capacity, and stenosis at the posterior cerebral arteries.

Asymptomatic MMD patients are considered to have not a low incidence of stroke [7, 20, 38, 56]. In our recent study [7], 35 adult patients with asymptomatic MMD showed 2.5%/person-year of hemorrhage and 0.8% of ischemic stroke. Five-year stroke rate was about 15%, and asymptomatic patients showed best clinical course, compared to the patients with hemorrhagic or ischemic presentations. Kuroda et al. analyzed that 40 patients presented with 3.2%/person-year of stroke for a mean follow-up of 43.7 months, in which hemorrhage and infarction each were 2.4% and 0.8% [38]. Twenty-one percent of the patients eventually experienced

neurological symptoms; however, 97.5% of them showed tolerable neurological status (modified Rankin score of 2 or less). Yang et al. showed 4% of hemorrhage and 1.3% of ischemia in 42 patients with 75 hemispheres during the mean 37.3 months [56]. Risk factor of clinical deterioration was solely the preoperative decrease in cerebrovascular reserve capacity. Jo et al.'s report showed 7.5% of transient ischemic attack and none with infarction and hemorrhage in 40 adults with 74 hemispheres for about 32 months [20]. Risk factors included smoking and compromise in preoperative reserve capacity. Therefore, routine follow-up should be performed in patients with asymptomatic presentation, especially in those with risk factors.

10.1.2 Hemorrhagic MMD

Majority of pediatric patients present with ischemic symptoms, and about 3% of them is known to manifest cerebral hemorrhage [3]. Meanwhile, adults present with cerebral hemorrhage in about 20–30% [7]. Generally, intraventricular hemorrhage is more common than intracerebral hemorrhage [30], and bleeding risk factors include cerebral microbleeds, older age, presence of prominent anterior choroidal, and posterior communicating arteries [24, 36, 43–45, 52].

It is well known that hemorrhagic MMD usually has a poor clinical course if untreated [7, 36, 42, 44]. According to Kobayashi et al.'s study, 33.3% of the patients experienced rebleeding for a mean follow-up of 80.6 months, recovery rate after rebleeding decreased into half of that after initial bleeding, and mortality rate increased four times [36]. Morioka et al. reported that rebleeding occurred in 61.1% of the patients during 12.7 years, and 77.3% of the patients with rebleeding were clinically aggravated [44]. Presenting age of the initial bleeding 36 years or more was a risk factor of rebleeding. In a recent prospective study from Japan [42], rebleeding rate was 34.2% in patients untreated for 5 years. As the follow-up period was longer and patients were older, rebleeding rate was considered to increase. A risk factor of rebleeding in the same patient group was analyzed as the initial posterior hemorrhage which was defined as one attributable to perforating arteries from the posterior cerebral artery or choroidal arteries, including those located in the thalamus, posterior half of the temporal lobe, parietal lobe, occipital lobe, subependymal area of the posterior part of the lateral ventricle including the trigon, or posterior half of the corpus callosum [53].

10.1.3 Fate of Unilateral MMD

Previously, unilateral involvement with moyamoya vessels and unknown causes was defined as probable MMD. As the cases of unilateral involvement increase and bilateral progression is well known [16, 18, 21, 25, 27, 28, 39, 41, 47, 57], however, unilateral involvement in children became definitive MMD in the 2012 Japan

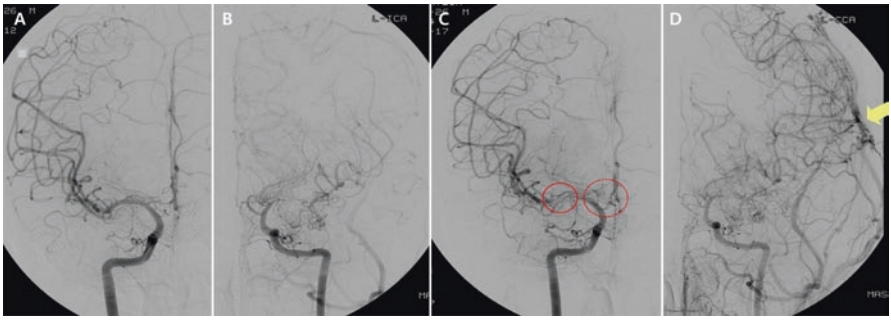

Fig. 10.1 Unilateral to bilateral progression. A young adult female presented with ischemic symptoms related to *left-sided* hemisphere. Preoperative cerebral angiography showed steno-occlusion of left distal ICA and proximal ACA and MCA (**b**) and stenosis of *right* ACA (**a**). On 8-month follow-up angiography after *left* combined bypass surgery, new revascularization was abundant at the *left* MCA territory (**d**); however, new progression of severe stenosis at the *right* proximal MCA and ACA was identified (**c**). Figure is available in color online only

guideline [50]. Incidence of unilateral MMD is about 8.5–20%, 0–58.8% of which is reported to progress into the bilateral disease [16, 18, 25, 39, 41, 47, 57]. The reason why the incidences of bilateral progression are widely various is thought to be related with the various range of follow-up duration from 13 to 73 months and the different ethnicity. Prognostic factors of bilateral progression included vascular abnormality contralateral to the involved side of internal carotid artery, female, younger age, and family history [25, 39, 41, 47, 57]. Progression of bilateral involvement as well as disease stage is known to be more rapid and commonly occur in pediatrics than in adults. However, progression in adult patients is recently thought as frequent as in pediatrics [39, 41]. Therefore, patients with unilateral involvement should be managed based on the clinical symptoms and diagnostic results. Moreover, routine imaging follow-up is needed (Fig. 10.1).

10.2 Surgical Outcomes

10.2.1 Ischemic MMD

Surgical outcomes in ischemic MMD are satisfactory. Most of pediatric cases were treated with indirect bypass surgery, with a stroke risk of 0.2–1.6%/year after surgery [31]. In addition, most of them surgically treated grew up well and had a normal life [48]. On the other hand, there has been a mood of superiority of direct/combined bypass surgery over indirect procedure in adult patients even though there is no high level of evidences. The stroke risk is known to be 0–1.6%/year after direct/combined bypass surgery and 0–14.3%/year after indirect bypass [34].

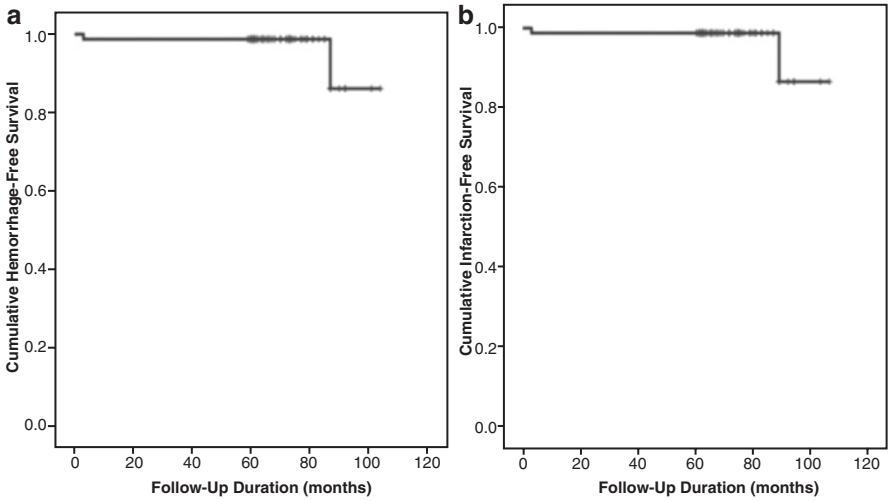

Fig. 10.2 Kaplan-Meier survival curves of hemorrhage (**a**) and infarction (**b**). The 5-year event-free survival rates for symptomatic hemorrhage and infarction ipsilateral to the operated hemispheres were all 98.7% (Reproduced from Cho et al. [8] under the permission by Wolters Kluwer Health, Inc.)

10.2.1.1 Surgery vs Conservative Management

Few comparative studies about the conservation and surgery exist. Choi et al.'s observation study about pediatric MMD said that 57% of the patients with conservative management became aggravated; however, 14% of those who got surgery became worse [11]. According to a recent meta-analysis comparing surgery to conservative management in all aged patients with MMD [49], stroke risk was significantly lower in surgery group than conservative group. In our long-term surgical outcome in terms of clinical, angiographic, and hemodynamic aspects [8], all the aspects continued to improve till 6 months after surgery and thereafter became stationary in 5 years. Five-year infarction- and hemorrhage-free survival rates were 98.7% each, and infarction and hemorrhagic risks were 0.2% and 0.4%/person-year, respectively (Fig. 10.2). Considering the natural history of hemodynamically stable MMD patients in our institution as 2.2%/person-year of infarction and 2.3% of hemorrhage (Fig. 10.3) [7], stroke recurrence rate is much lower in surgery group than in observation group although the surgery group had worse preoperative conditions than observation group. Merely, permanent postoperative complication rate was 3.9%, for which a few years would be needed to overcome the natural history of conservative group. Recently, our affiliated group retrospectively compared direct/combined bypass surgery group of 301 adults in their institution to observation group of 140 patients in our institution [35]. One- and 5-year ischemic stroke rates did not differ; however, 10-year rate was superior in surgery group than observation group (3.9% versus 13.3%). In addition, the only independent protective factor was bypass surgery. Postoperative stroke complication rate in this study was

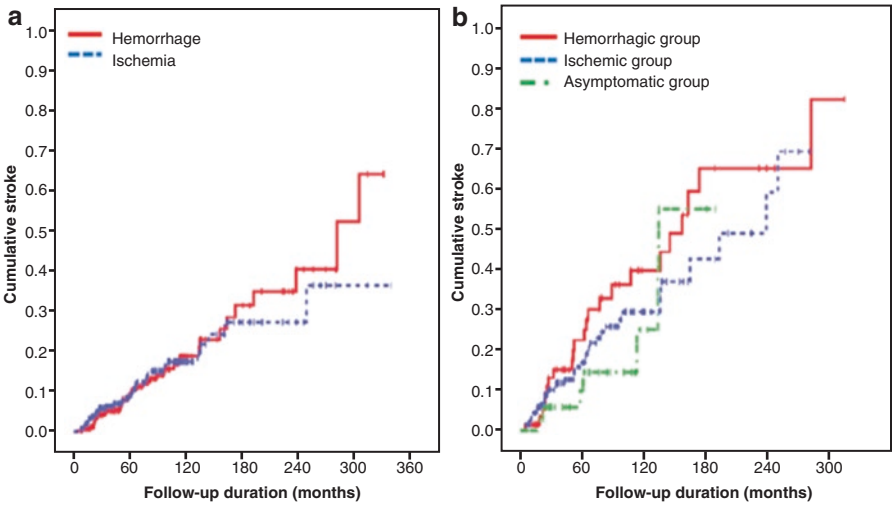

Fig. 10.3 Kaplan-Meier curves of stroke events during conservative follow-up. There was no significant difference between hemorrhagic and ischemic strokes in all patients (p = 0.857) (**a**). Among the groups with different clinical presentations, there was no significant difference in overall stroke events (p = 0.461) (**b**). Figure is available in color online only (Modified from Cho et al. [7] under the permission by American Association of Neurological Surgery)

2.7%, and more than 5 years of time period was needed for surgical benefit to overcome the natural history of observation group (Fig. 10.4). So, complication rate of each institution's own as well as patients' condition and age should be considered before deciding the surgery.

10.2.1.2 Direct/Combined vs Indirect Bypass Surgery

According to some comparative studies between direct/combined and indirect bypass surgeries in adult MMD [1, 4, 8, 26], overall neurological outcomes are similarly satisfactory; however, detailed clinical outcomes were better, and angiographic areas were wider in direct/combined bypass than indirect procedures. A recent meta-analysis showed that long-term prevention of hemorrhage and ischemia and favorable clinical outcomes were better in direct/combined procedures, although overall complication rates were not different [51]. Kazumata et al.'s systemic review analyzed that there was no difference in postoperative stroke complication between direct/combined and indirect surgeries (7.6% versus 5.1%, respectively); however, neorevascularization was significantly better in direct/combined procedures [23]. Moreover, comparing some patients with long-term follow-up of about 4 years, recurrent stroke rate was higher in indirect bypass surgery (11.2% versus 3.5%). We once analyzed the short-term outcomes of combined and indirect bypasses although unpublished, in which clinical and hemodynamic outcomes were similar; however,

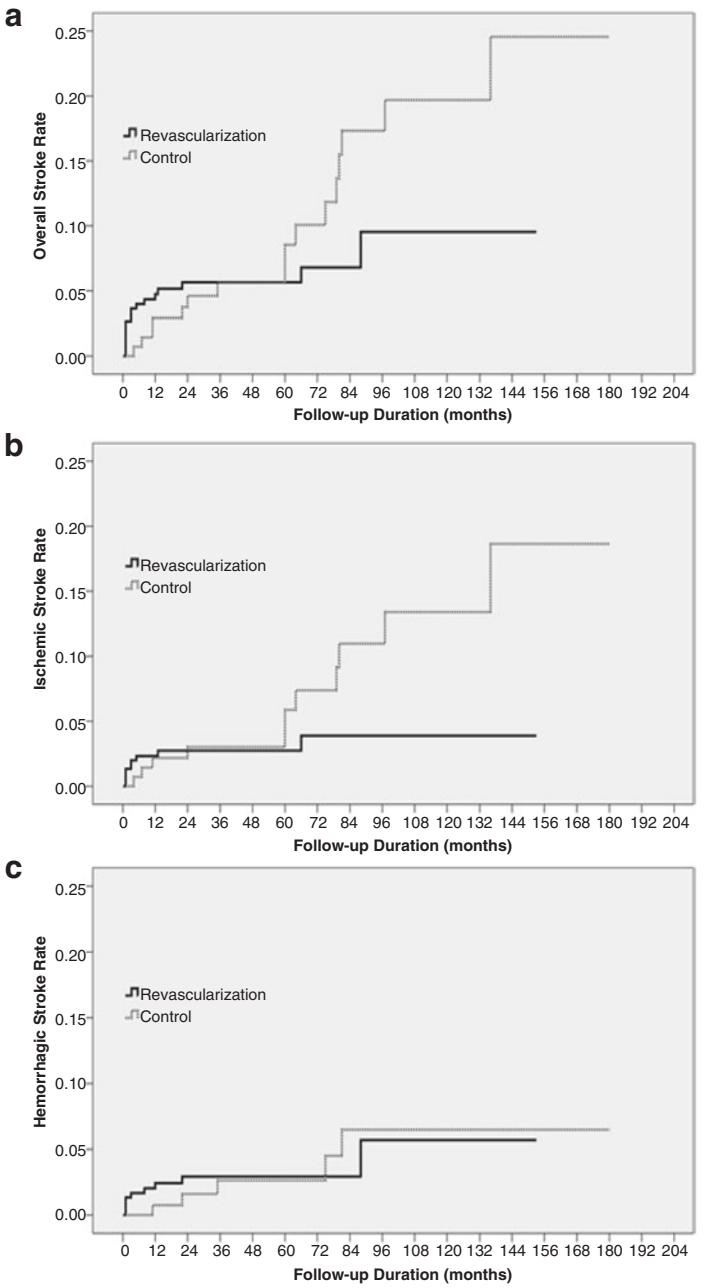

Fig. 10.4 Survival curves of any stroke (**a**), ischemic stroke (**b**), and hemorrhagic stroke (**c**). A steep slope shortly after 0 month means perioperative stroke events. In around 5 and 7 years after surgery, natural course in surgery group becomes better than that in observation group (Modified from Kim et al. [36] under the permission by American Association of Neurological Surgery)

revascularization area was significantly wider in combined bypass group. Which clinical impact would be had on by wider revascularization after combined bypass surgery is needed to be observed for a longer period. As most of cases reported on papers are small numbers and different in basal characteristics, evaluation modalities, surgical skills, and follow-up period, which kind of bypass surgery be more advantageous is still hard to determine. Merely, considerable exercise and experience are needed to perform direct bypass surgery. Thus, if direct bypass is not available, indirect bypass surgery can be a good alternative.

10.2.2 Hemorrhagic MMD

Bypass surgery for ischemic MMD is well known to be effective in both pediatric and adult patients [34]. Meanwhile, the efficacy of bypass surgery in preventing from rebleeding in hemorrhagic MMD has been controversial. A 10-year prospective study concluded that there was no significant difference in rebleeding risk between surgery and non-surgery groups [55]. A recent meta-analysis showed the protective effect of bypass surgery on the rebleeding [49]. According to a multicenter prospective and randomized trial from Japan [42], surgery group showed significantly lower incidence of rebleeding than non-surgery group (14.3% versus 34.2%). Annual rebleeding rate was 3.2%/year in surgery group and 8.2%/year on conservative group. This trial is the only prospective and randomized study, and there was no dropout case during follow-up. However, the result is not considered to be complete because the statistical significance varied according to the statistical methods. So, further study is needed.

10.2.3 Cerebral Hyperperfusion Syndrome (CHS) and Other Complications

Postoperative complications include infarction, hemorrhage, cerebral hyperperfusion syndrome, wound problem, infection, and so on. Complication rate is reported in about 1.6–16%, among which permanent ischemic complication is 0.9–8% and permanent hemorrhagic one is 0.7–8%, and the occurrence is more common in adult patients [23, 34]. Noh et al. reported that 50% of ischemic stroke occurred within 1 month after surgery and 1- and 5-year stroke recurrence rates were 24.4% each [46]. In our long-term surgical outcomes [8], 5-year infarction and hemorrhage risks were 1.3% each; however, permanent perioperative neurological complication rate was 6.7%. Therefore, perioperative management to prevent or recover from complications is a critical step to improve the surgical outcomes.

CHS is well documented in patients with carotid stenosis, and it was also identified in adult patients with MMD after low-flow direct bypass surgery [12, 29].

In our previous report, the incidence of clinical CHS was identified in 17% of the patients, and cerebral hyperperfusion became maximal around in the third day after surgery [29]. The presumptive pathomechanism is transient dysfunction of cerebral autoregulation: autoregulatory function has been chronically impaired, and clinical symptoms corresponding to the hyperperfusion areas by direct bypass pedicle can occur until the recovery of its function. CHS is reported to occur in about 21.5–50% after direct bypass surgery [34], and various clinical symptoms manifest from headache to neurological deficits and intracerebral hemorrhage. We experienced similar situations even after indirect bypass surgery for adult MMD [10]. Hyperperfusion corresponding to the clinical symptoms was identified on brain single-photon emission computed tomography. It is not clear whether this was real hyperperfusion or manifestation by different mechanisms; however, similar suspicion was also identified in pediatric cases after indirect bypass surgery. So, we plan to keep a close watch about it. When CHS is suspicious after combined bypass surgery in our institution, brain computed tomography + diffusion-weighted magnetic resonance (MR) imaging or MR imaging with diffusion-weighted images + susceptibility-weighted images + arterial spin labelling images are acquired to differentially diagnose hemorrhage, infarction, and CHS. After CHS is diagnosed, protocolized medical management is started: normotension or slight hypotension within −10% of the usual systolic blood pressure, normohydration, and the transient use of steroid.

10.2.4 Other Surgical Considerations

10.2.4.1 Coverage of Other Territories Beyond the Middle Cerebral Artery (MCA) Territory by Bypass Surgery at the Cortical Branches of MCA

There are many kinds of surgical skills for MMD which are as follows: direct/combined bypass and indirect bypass; single- and double-barrel direct bypass; encephaloduroarterio-, encephalgaleo-, and encephalomyo-synangiosis as indirect bypass; and multiple burr hole trephination. Irrespective of the surgical skills, most of them are performed at the MCA territory because MCA territory is the widest, most of clinical symptoms are related with MCA, surgery at the MCA territory is feasible, and surgical outcomes have been satisfactory. Rarely, bypass surgery at the other territories, such as anterior (ACA) and posterior cerebral arteries (PCA), has been reported on occasion [15, 22]. Except for some cases necessitating other kinds of bypass surgery, our institution has generally performed superficial temporal artery-MCA single pedicle microanastomosis and encephalodurogaleosynangiosis, resulting in satisfactory outcomes [8]. Recently, we found out that combined bypass surgery at the MCA territory could improve the ACA as well as MCA-related hemodynamic and clinical states (Fig. 10.5) [9]. Such phenomenon was more prominent in patients with preoperative symptoms related to ACA territory. According to a systemic review [23] and our unpublished data, revascularization area seems to be

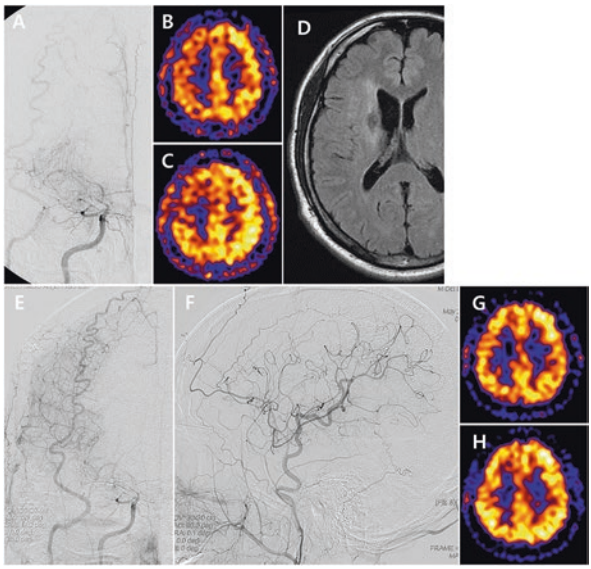

Fig. 10.5 A case of 35-year adult MMD patient treated with right-sided combined bypass surgery (superficial temporal artery angular branch of middle cerebral artery single direct bypass + encephalogaleosynangiosis). Preoperative evaluation exhibited near occlusion of right distal ICA with limited basal moyamoya vessels on cerebral angiography (**a**), decrease in basal perfusion (**b**) and reserve capacity (**c**) of *right* ICA territory on Diamox® single-photon emission computed tomography, and subacute infarction at the *right* corona radiata on MR imaging (**d**). On 6-month follow-up work-up, abundant revascularization dominantly by direct pedicle in the territories of ACA as well as MCA (**e** and **f**) was demonstrated with a finding of regression of the *right* distal ICA and basal moyamoya vessels (**e**). In addition, basal perfusion (**g**) and reserve capacity (**h**) much improved

wider in combined bypass surgery than in indirect procedure. Although previous studies failed to show a significant difference in clinical outcomes between combined/direct and indirect bypass surgeries [1, 4, 8, 23, 26, 51], combined bypass surgery is thought to be more beneficial in cases necessitating wider flow augmentation, for example, accompanying ACA or PCA as well as MCA-related symptoms, and considering the cognition as an important function of medial frontal lobe. In the same vein, double-barrel direct bypass surgery with indirect procedures may be more effective than single direct bypass in patients with hemodynamic impairment beyond the MCA territory. Kim et al. compared two different surgical skills (indirect bypass at MCA territory alone versus indirect bypass at MCA and ACA territories) in pediatric patients [33]. Additional indirect bypass at the ACA territory significantly improved ACA-related symptoms and surgical outcomes in terms of hemodynamic and angiographic states. Considering case-by-case conditions, personalized surgical strategies would be more helpful.

10.2.4.2 Role of Direct and Indirect Bypasses in the Combined Surgery

The roles of indirect flap and direct pedicle in the combined bypass surgery have long been discussed. One opinion is that direct pedicle would play a dominant role for flow augmentation, and the other is that indirect flap would become dominant and direct pedicle slowly regress [8, 17, 54]. In our previous study [8], revascularization area continued to widen until 5 years after surgery, while dominant, complementary, and poor developments of direct or indirect components were various and the patterns changed over time. One of the interesting findings was that the incidence of occlusion of direct pedicle was 6.6% in 6 months after surgery and 23% in 5 years. So, it was speculated that direct pedicle would play a role of acute flow augmentation in the early phase and indirect flap would replace the areas in which blood flow from direct pedicle could not reach because of the progressive occlusion of the intracranial arteries (named compartmentation) in the later phase. This hypothesis is indirectly confirmed also in Uchino et al.'s recent report [54], in which dominancy of indirect and direct bypass differed in pediatric and adult patients; however, each component of indirect and direct bypass showed reciprocal roles, and development of indirect bypass was significant in patients with advanced stages of MMD (Suzuki grade 4 or more). Therefore, direct and indirect bypasses seem to play complementary roles according to the temporal and spatial situations. In that sense, combined bypass surgery may be superior to direct or indirect procedures alone.

10.3 Medical Management

Angiographic feature of MMD is the progressive steno-occlusion of the distal internal carotid artery and proximal anterior and middle cerebral arteries. Histologic feature is the intimal thickening by proliferation of smooth muscle cells with no inflammatory/atheromatous changes. The cause of MMD is still idiopathic, and it is different from the intracranial atherosclerotic stenosis or vasculitis accompanying endothelial damage. So, there have been no fundamental treatment modalities; instead, symptomatic medical treatment and bypass surgery for flow augmentation have been performed. Among the medical treatment, the use of antiplatelets has been controversial. Because the main pathophysiology in MMD is the hemodynamic insufficiency following steno-occlusion of the intracranial vessels, drugs, such as antiplatelets inhibiting platelet adhesion/aggregation and statin stabilizing the plaque, are traditionally considered not effective. However, microthromboembolism is reported to occur even in MMD, and it was closely related with the development of ischemic events [5]. In addition, atherosclerotic vessel change can be superimposed as adult patients get older, and drugs can be selectively needed in order to maintain the patency at the bypass site. According to a recent Kraemer et al.'s report, about 31% of the responders used antiplatelets for the MMD patients [37]. Interestingly, Asian experts tended to prescribe antiplatelets less frequently

than Western doctors, the reason of which was speculated that Asian patients show higher incidence of hemorrhagic presentation. Actually, there is ethnic difference in hemorrhagic presentation: less than 15% in Western countries, 29% in Hawaii where the proportion of Asian inhabitants is high, and 30–60% in East Asian countries [2, 7]. A recent prospective study reported that ischemic events did not differ between antiplatelet and non-antiplatelet groups, and hemorrhagic events were higher in the non-antiplatelet group than in the antiplatelet group [55]. Further studies are needed to make a conclusion in the use of antiplatelets.

References

1. Abla AA, Gandhoke G, Clark JC, Oppenlander ME, Velat GJ, Zabramski JM, et al. Surgical outcomes for moyamoya angiopathy at barrow neurological institute with comparison of adult indirect encephaloduroarteriosynangiosis bypass, adult direct superficial temporal artery-to-middle cerebral artery bypass, and pediatric bypass: 154 revascularization surgeries in 140 affected hemispheres. Neurosurgery. 2013;73:430–9.
2. Acker G, Goerdes S, Schneider UC, Schmiedek P, Czabanka M, Vajkoczy P. Distinct clinical and radiographic characteristics of moyamoya disease amongst European Caucasians. Eur J Neurol. 2015;22:1012–7.
3. Ahn JH, Wang KC, Phi JH, Lee JY, Cho BK, Kim IO, et al. Hemorrhagic moyamoya disease in children: clinical features and surgical outcome. Childs Nerv Syst. 2012;28:237–45.
4. Bang JS, Kwon OK, Kim JE, Kang HS, Park H, Cho SY, Oh CW. Quantitative angiographic comparison with the OSIRIS program between the direct and indirect revascularization modalities in adult moyamoya disease. Neurosurgery. 2012;70:625–32.
5. Chen J, Duan L, Xu WH, Han YQ, Cui LY, Gao S. Microembolic signals predict cerebral ischaemic events in patients with moyamoya disease. Eur J Neurol. 2014;21:785–90.
6. Chiu D, Shedden P, Bratina P, Grotta JC. Clinical features of moyamoya disease in the United States. Stroke. 1998;29:1347–51.
7. Cho WS, Chung YS, Kim JE, Jeon JP, Son YJ, Bang JS, et al. The natural clinical course of hemodynamically stable adult moyamoya disease. J Neurosurg. 2015;122:82–9.
8. Cho WS, Kim JE, Kim CH, Ban SP, Kang HS, Son YJ, et al. Long-term outcomes after combined revascularization surgery in adult moyamoya disease. Stroke. 2014;45:3025–31.
9. Cho WS, Kim JE, Paeng JC, Suh M, Kim Y, Kang HS, et al. Can combined bypass surgery at middle cerebral artery territory save anterior cerebral artery territory in adult moyamoya disease? Neurosurgery. 2016 July 25. [Epub ahead of print]
10. Cho WS, Lee HY, Kang HS, Kim JE, Bang JS, Oh CW. Symptomatic cerebral hyperperfusion on SPECT after indirect revascularization surgery for Moyamoya disease. Clin Nucl Med. 2013;38:44–6.
11. Choi JU, Kim DS, Kim EY, Lee KC. Natural history of moyamoya disease: comparison of activity of daily living in surgery and non surgery groups. Clin Neurol Neurosurg. 1997;99(Suppl 2):S11–8.
12. Fujimura M, Kaneta T, Mugikura S, Shimizu H, Tominaga T. Temporary neurologic deterioration due to cerebral hyperperfusion after superficial temporal artery-middle cerebral artery anastomosis in patients with adult-onset moyamoya disease. Surg Neurol. 2007;67:273–82.
13. Gross BA, Du R. The natural history of moyamoya in a North American adult cohort. J Clin Neurosci. 2013;20:44–8.
14. Hallemeier CL, Rich KM, Grubb Jr RL, Chicoine MR, Moran CJ, Cross III DT, et al. Clinical features and outcome in North American adults with moyamoya phenomenon. Stroke. 2006;37:1490–6.

15. Hayashi T, Shirane R, Tominaga T. Additional surgery for postoperative ischemic symptoms in patients with moyamoya disease: the effectiveness of occipital arteryposterior cerebral artery bypass with an indirect procedure: technical case report. Neurosurgery. 2009;64:E195–6.
16. Hayashi K, Suyama K, Nagata I. Clinical features of unilateral moyamoya disease. Neurol Med Chir (Tokyo). 2010;50:378–85.
17. Houkin K, Yoshimoto T, Kuroda S, Ishikawa T, Takahashi A, Abe H. Angiographic analysis of moyamoya disease – how does moyamoya disease progress? Neurol Med Chir (Tokyo). 1996;36:783–7.
18. Ikezaki K, Inamura T, Kawano T, Fukui M. Clinical features of probable moyamoya disease in Japan. Clin Neurol Neurosurg. 1997;99(Suppl 2):S173–7.
19. Imaizumi T, Hayashi K, Saito K, Osawa M, Fukuyama Y. Long-term outcomes of pediatric moyamoya disease monitored to adulthood. Pediatr Neurol. 1998;18:321–5.
20. Jo KI, Yeon JY, Hong SC, Kim JS. Clinical course of asymptomatic adult moyamoya disease. Cerebrovasc Dis. 2014;37:94–101.
21. Kawano T, Fukui M, Hashimoto N, Yonekawa Y. Follow-up study of patients with "unilateral" moyamoya disease. Neurol Med Chir (Tokyo). 1994;34:744–7.
22. Kawashima A, Kawamata T, Yamagucho K, Hori T, Okada Y. Successful superficial temporal artery-anterior cerebral artery direct bypass using a long graft for moyamoya disease: technical note. Neurosurgery. 2010;67(3 suppl operative):ons145–9.
23. Kazumata K, Ito M, Tokairin K, Ito Y, Houkin K, Nakayama N, et al. The frequency of postoperative stroke in moyamoya disease following combined revascularization: a single-university series and systematic review. J Neurosurg. 2014;121:432–40.
24. Kazumata K, Shinbo D, Ito M, Shichinohe H, Kuroda S, Nakayama N, et al. Spatial relationship between cerebral microbleeds, moyamoya vessels, and hematoma in moyamoya disease. J Stroke Cerebrovasc Dis. 2014;23:1421–8.
25. Kelly ME, Bell-Stephens TE, Marks MP, Do HM, Steinberg GK. Progression of unilateral moyamoya disease: a clinical series. Cerebrovasc Dis. 2006;22:109–15.
26. Kim DS, Huh PW, Kim HS, Kim IS, Choi S, Mok JH, et al. Surgical treatment of moyamoya disease in adults: combined direct and indirect vs. indirect bypass surgery. Neurol Med Chir (Tokyo). 2012;52:333–8.
27. Kim JE, Jeon JS. An update on the diagnosis and treatment of adult moyamoya disease taking into consideration controversial issues. Neurol Res. 2014;36:407–16.
28. Kim JE, Kim KM, Kim JG, Kang HS, Bang JS, Son YJ, et al. Clinical features of adult moyamoya disease with special reference to the diagnosis. Neurol Med Chir (Tokyo). 2012;52:311–7.
29. Kim JE, Oh CW, Kwon OK, Park SQ, Kim SE, Kim YK. Transient hyperperfusion after superficial temporal artery/middle cerebral artery bypass surgery as a possible cause of postoperative transient neurological deterioration. Cerebrovasc Dis. 2008;25:580–6.
30. Kim JS. Moyamoya disease: epidemiology, clinical features, and diagnosis. J Stroke. 2016;18:2–11.
31. Kim SK, Cho BK, Phi JH, Lee JY, Chae JH, Kim KJ, et al. Pediatric moyamoya disease: an analysis of 410 consecutive cases. Ann Neurol. 2010;68:92–101.
32. Kim SK, Seol HJ, Cho BK, Hwang YS, Lee DS, Wang KC. Moyamoya disease among young patients: its aggressive clinical course and the role of active surgical treatment. Neurosurgery. 2004;54:840–4.
33. Kim SK, Wang KC, Kim IO, Lee DS, Cho BK. Combined encephaloduroarteriosynangiosis and bifrontal encephalogaleo (periosteal) synangiosis in pediatric moyamoya disease. Neurosurgery. 2002;50:88–96.
34. Kim T, Oh CW, Bang JS, Kim JE, Cho WS. Moyamoya disease: treatment and outcomes. J Stroke. 2016;18:21–30.

35. Kim T, Oh CW, Kwon OK, Hwang G, Kim JE, Kang HS, et al. Stroke prevention by direct revascularization for patients with adult-onset moyamoya disease presenting with ischemia. J Neurosurg. 2016;124:1788–93.
36. Kobayashi E, Saeki N, Oishi H, Hirai S, Yamaura A. Long-term natural history of hemorrhagic moyamoya disease in 42 patients. J Neurosurg. 2000;93:976–80.
37. Kraemer M, Berlit P, Diesner F, Khan N. What is the expert's option on antiplatelet therapy in moyamoya disease? Results of a worldwide Survey. Eur J Neurol. 2012;19:163–7.
38. Kuroda S, Hashimoto N, Yoshimoto T, Iwasaki Y. Radiological findings, clinical course, and outcome in asymptomatic moyamoya disease: results of multicenter survey in Japan. Stroke. 2007;38:1430–5.
39. Kuroda S, Ishikawa T, Houkin K, Nanba R, Hokari M, Iwasaki Y. Incidence and clinical features of disease progression in adult moyamoya disease. Stroke. 2005;36:2148–53.
40. Lee JY, Phi JH, Wang KC, Cho BK, Shin MS, Kim SK. Neurocognitive profiles of children with moyamoya disease before and after surgical intervention. Cerebrovasc Dis. 2011;31:230–7.
41. Lee SC, Jeon JS, Kim JE, Chung YS, Ahn JH, Cho WS, et al. Contralateral progression and its risk factor in surgically treated unilateral adult moyamoya disease with a review of pertinent literature. Acta Neurochir. 2014;156:103–11.
42. Miyamoto S, Yoshimoto T, Hashimoto N, Okada Y, Tsuji I, Tominaga T, et al. Effects of extracranial-intracranial bypass for patients with hemorrhagic moyamoya disease: results of the Japan adult moyamoya trial. Stroke. 2014;45:1415–21.
43. Morioka M, Hamada J, Kawano T, Todaka T, Yano S, Kai Y, et al. Angiographic dilatation and branch extension of the anterior choroidal and posterior communicating arteries are predictors of hemorrhage in adult moyamoya patients. Stroke. 2003;34:90–5.
44. Morioka M, Hamada J, Todaka T, Yano S, Kai Y, Ushio Y. High-risk age for rebleeding in patients with hemorrhagic moyamoya disease: long-term follow-up study. Neurosurgery. 2003;52:1049–54.
45. Nah HW, Kwon SU, Kang DW, Ahn JS, Kwun BD, Kim JS. Moyamoya disease-related versus primary intracerebral hemorrhage: location and outcomes are different. Stroke. 2012;43:1947–50.
46. Noh HJ, Kim SJ, Kim JS, Hong SC, Kim KH, Jun P, et al. Long term outcome and predictors of ischemic stroke recurrence in adult moyamoya disease. J Neurol Sci. 2015;359:381–8.
47. Park EK, Lee YH, Shim KW, Choi JU, Kim DS. Natural history and progression factors of unilateral moyamoya disease in pediatric patients. Childs Nerv Syst. 2011;27:1281–7.
48. Phi JH, Wang KC, Cho BK, Lee MS, Lee JH, Yu KS, et al. Long-term social outcome in children with moyamoya disease who have reached adulthood. J Neurosurg Pediatr. 2011;8:303–9.
49. Qian C, Yu X, Li J, Chen J, Wang L, Chen G. The efficacy of surgical treatment for the secondary prevention of stroke in symptomatic moyamoya disease: a meta-analysis. Medicine (Baltimore). 2015;94:e2218.
50. Research Committee on the Pathology and Treatment of Spontaneous Occlusion of the Circle of Willis, Health Labour Sciences Research Grant for Research on Measures for Intractable Diseases. Guidelines for diagnosis and treatment of moyamoya disease (spontaneous occlusion of the circle of Willis). Neurol Med Chir (Tokyo). 2012;52:245–66.
51. Sun H, Wilson C, Ozpinar A, Safavi-Abbasi S, Zhao Y, Nakaji P, et al. Perioperative complications and long-term outcomes after bypasses in adults with moyamoya disease: a systematic review and meta-analysis. World Neurosurg. 2016; doi:10.1016/j.wneu.2016.04.083.
52. Sun W, Yuan C, Liu W, Li Y, Huang Z, Zhu W, et al. Asymptomatic cerebral microbleeds in adult patients with moyamoya disease: a prospective cohort study with 2 years of follow-up. Cerebrovasc Dis. 2013;35:469–75.
53. Takahashi JC, Funaki T, Houkin K, Inoue T, Ogasawara K, Nakagawara J, et al. Significance of the hemorrhagic site for recurrent bleeding: prespecified analysis in the Japan adult moyamoya trial. Stroke. 2016;47:37–43.

54. Uchino H, Kim JH, Fujima N, Kazumata K, Ito M, Nakayama N, et al. Synergistic interactions between direct and indirect bypasses in combined procedures: the significance of indirect bypasses in moyamoya disease. Neurosurgery. 2016; doi:10.1227/NEU.0000000000001201.
55. Yamada S, Oki K, Itoh Y, Kuroda S, Houkin K, Tominaga T, et al. Effects of surgery and antiplatelet therapy in ten-year follow-up from the registry study of research committee on moyamoya disease in Japan. J Stroke Cerebrovasc Dis. 2016;25:340–9.
56. Yang J, Hong JC, Oh CW, Kwon OK, Hwang G, Kim JE, et al. Clinicoepidemiological features of asymptomatic moyamoya disease in adult patients. J Cerebrovasc Endovasc Neurosurg. 2014;16:241–6.
57. Yeon JY, Shin HJ, Kong DS, Seol HJ, Kim JS, Hong SC, et al. The prediction of contralateral progression in children and adolescents with unilateral moyamoya disease. Stroke. 2011;42:2973–6.

Chapter 11
Significance of *RNF213* in Clinical Management in Japan

Yohei Mineharu, Yasushi Takagi, and Susumu Miyamoto

Abstract *RNF213* is a main susceptibility gene for moyamoya disease. Worldwide, many mutations of *RNF213* have been reported in association with moyamoya disease. Among these, p.R4810K is the most frequent variant and is found in approximately 95% of patients with familial moyamoya disease and in approximately 80% of sporadic cases in Japan. The variant is strongly associated with moyamoya disease, with an odds ratio of nearly 200, but caution is necessary when it is used in clinical practice because there are approximately 2% of p.R4810K carriers who do not develop moyamoya disease in the general population. The clinical impact of the p.R4810K variant has been assessed by recent studies. Patients who are homozygous for the p.R4810K variant had an earlier onset age (mostly under the age of 4 years), and there was wider distribution (involvement of bilateral hemispheres and posterior circulation) and a more severe form of the disease with this variant. A contribution of *RNF213* to various types of intracranial steno-occlusive disease including unilateral moyamoya disease, quasi-moyamoya disease, and other intracranial steno-occlusive disease was also reported. In this chapter, we will summarize genetic characteristics of moyamoya disease including familial cases and discuss clinical application of *RNF213* genotyping in patients with moyamoya disease and other intracranial steno-occlusive diseases.

Keywords *RNF213* • Familial moyamoya disease • Low penetrance • Genetic diagnosis • Moyamoya spectrum

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11.1 Introduction

RNF213 has been recognized as a main susceptibility gene for moyamoya disease [1, 2]. This gene is also important in other intracranial steno-occlusive diseases [3, 4]. Although the gene plays an important role, *RNF213* used for genetic testing is not sufficient alone to make a diagnosis or to fully understand the pathophysiology of the disease. Clarification of other factors, including environmental and other genetic factors, in the development of moyamoya disease is essential. In Japan as well as in Korea and China, p.R4810K is the most frequent variant of *RNF213* that is associated with moyamoya disease, and it has the potential to be used for genetic diagnosis [1, 2, 5–13].

11.2 Genetics of Moyamoya Disease

To understand the genetics of moyamoya disease and the clinical significance of *RNF213*, it is essential to understand the characteristics of familial moyamoya disease (Fig. 11.1a–d) [3, 14]. There are five main characteristics of familial moyamoya disease, which are (1) the inheritance pattern is autosomal dominant with low penetrance; (2) a variety of phenotypes are seen in a single family including moyamoya disease, unilateral moyamoya disease, and steno-occlusive lesions around the terminal portion of the internal carotid arteries (ICA) without apparent moyamoya vessels; (3) females are predominantly affected, with a female-to-male ratio of 2.71:1; (4) affected twins are mostly identical and female, and some show discordant phenotypes; and (5) affected mothers are more likely to produce female offspring with late-onset disease (adult-onset or asymptomatic), suggesting that genomic imprinting is involved in moyamoya disease.

11.2.1 Autosomal Dominant with Low Penetrance

The inheritance pattern of moyamoya disease is autosomal dominant with incomplete penetrance in most cases, although some patients might show a polygenic pattern. In 2006, Mineharu et al. examined the characteristics of 15 highly aggregated Japanese families with three or more affected members; in total, there were 52 patients enrolled, comprising 38 women and 14 men [14]. In all of these families, there were three or more generations without consanguinity, and all types of transmission, including father-to-son, were observed. Among 135 offspring of affected individuals, nearly half (59/135, 43.7%) were patients with moyamoya disease or obligate carriers (individuals who are clinically unaffected but who carry a genetic risk factor according to the family pedigree analysis), suggesting that the transmission

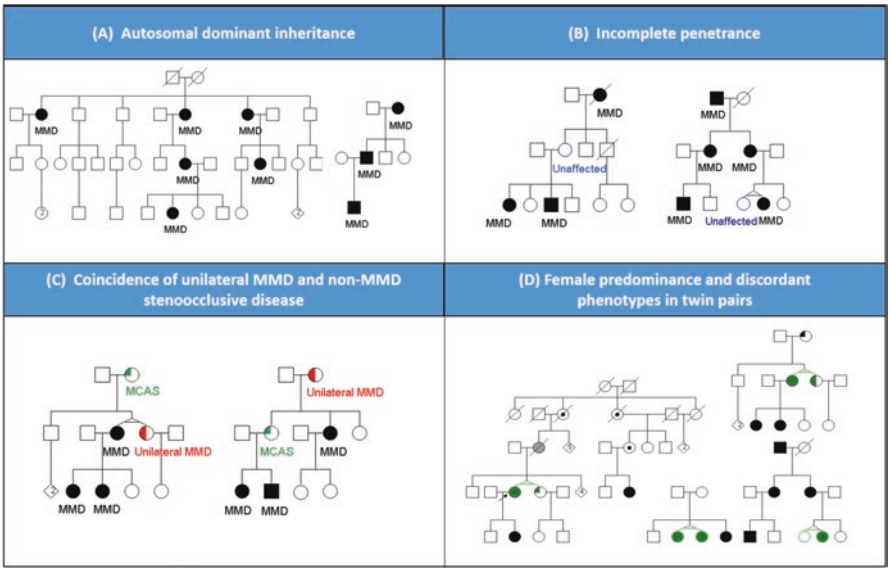


Fig. 11.1 Characteristics of familial moyamoya disease are helpful to determine the genetics of moyamoya disease and *RNF213* gene involvement. All patients with *filled*, *half-filled*, and *quarter-filled symbols* and obligate carriers with *dotted symbols* have the *RNF213* p. R4810K variant. (**a**) Patients in three consecutive generations indicate that the inheritance pattern of familial moyamoya disease is autosomal dominant. Both homozygous and heterozygous individuals have an increased risk of moyamoya disease. (**b**) Skipping a generation and discordant phenotypes in twin pairs suggest that penetrance of *RNF213* is incomplete. (**c**) *RNF213* p.R4810K is associated with moyamoya disease, unilateral moyamoya disease, and non-moyamoya intracranial steno-occlusive diseases such as middle cerebral artery stenosis. (**d**) Only females are affected in some families. Twin pairs are mostly female and identical.
Filled symbols indicate patients with moyamoya disease; *half-filled symbols* indicate unilateral moyamoya disease; *quarter-filled symbols* indicate intracranial steno-occlusive disease without moyamoya vessels; *MMD* moyamoya disease, *MCAS* middle cerebral artery stenosis

pattern was autosomal dominant with incomplete penetrance (Fig. 11.1a, b). Based on this assumption, parametric linkage analysis identified a disease locus at 17q25.3 [3], where the p.R4810K variant (c.14429G>A, rs112735431) in the *RNF213* gene was found to be associated with moyamoya disease [1, 2]. The homozygous (AA genotype; two risk alleles) and heterozygous (GA genotype; one risk allele) alleles of the p.R4810K variant were equally associated with moyamoya disease compared with the wild-type (GG genotype) control, and the odds ratio was as high as 300 using the dominant model [1, 2]. While p.R4810K was found in more than 80% of patients with moyamoya disease, the observation that the p.R4810K was found in about 2% of general population in East Asian countries [15] represents a low penetrance of this risk variant.

11.2.2 Moyamoya Disease Variant (Moyamoya Spectrum)

Pedigree analysis for familial moyamoya disease revealed that moyamoya disease and unilateral moyamoya disease or steno-occlusive lesions around the terminal portion of ICA without apparent moyamoya vessels (i.e., proximal middle cerebral artery stenosis) were present in a single family (Fig. 11.1c). These conditions were seen together in approximately 30% of families with familial moyamoya disease [3]. The occurrence of various phenotypes together in a single family suggests that these phenotypes may be a spectrum of moyamoya disease. Miyawaki et al. examined sporadic cases of intracranial steno-occlusive disease (mostly proximal middle cerebral artery stenosis or occlusion), and they reported that *RNF213* was found in approximately 25% of patients and that it was associated with moyamoya disease with an odds ratio of 16.8 [4, 16].

11.2.3 Female Predominance and Discordant Phenotypes

Patients with moyamoya disease are predominantly female, with a female-to-male ratio of 1.8–2.2:1 [17–19]. In familial moyamoya disease, for females to be affected predominantly was more prominent. The female-to-male ratio was 2.7:1 and the ratio of maternal transmission-to-paternal transmission was 3.44:1, showing maternal predominance, and mother-to-daughter transmission was most commonly observed (60.0%) [14]. Although underlying mechanisms are not known, most twin pairs with familial moyamoya disease are identical twins and female (Fig. 11.1d). Additionally, among the 12 monozygotic twins reported to date, five pairs showed discordant phenotypes (symptomatic vs. asymptomatic, bilateral vs. unilateral, or patients vs. non-patients) [14]. These findings suggest that genomic imprinting or epigenetic modification may be associated with moyamoya disease. Although recall bias may be associated with these phenomena, tissue-specific epigenetic studies such as methylation analysis may improve our understanding of moyamoya disease pathophysiology.

11.3 *RNF213*-Related Intracranial Steno-occlusive Disease

RNF213 was first identified as a susceptibility gene for moyamoya disease. A pedigree analysis and intensive genotyping and sequencing of *RNF213* showed that it is also associated with unilateral moyamoya disease and partially associated with intracranial steno-occlusive disease without moyamoya vessels and quasi-moyamoya disease. These diseases can be partly recognized as *RNF213*-related intracranial steno-occlusive disease (Fig. 11.2; Table 11.1A).

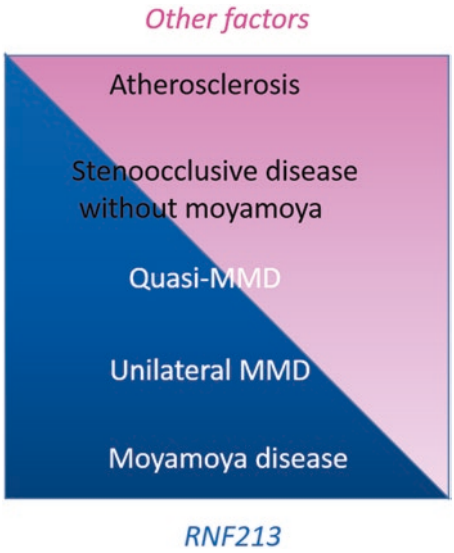


Fig. 11.2 Contribution of *RNF213* to the moyamoya spectrum
The contribution of *RNF213* is different among subtypes on the moyamoya spectrum including moyamoya disease, unilateral moyamoya disease, quasi-moyamoya disease, and non-moyamoya intracranial steno-occlusive disease (without moyamoya vessels). *RNF213* may also be associated with atherosclerosis. Other factors may include Down syndrome, neurofibromatosis type 1, or inflammatory disorders, but they have not yet been well clarified. Whether *RNF213* determines the therapeutic response to revascularization surgery remains unknown

11.3.1 Unilateral Moyamoya Disease

Unilateral moyamoya disease used to be regarded as probable moyamoya disease and was distinguished from (bilateral) moyamoya disease in adults. Moyamoya disease and unilateral moyamoya disease occurring together in a single family and identification of the p.R4810K variant as a common risk factor for both diseases influenced the current Guidelines of the Research Committee for Diagnosis of Moyamoya Disease in Japan, which recognizes both unilateral and bilateral lesions as definite moyamoya disease.

11.3.2 Intracranial Vascular Disease Without Moyamoya Vessels

As mentioned in Sect. 12.2.2, intracranial steno-occlusive disease around the terminal portion of the ICA without moyamoya vessels, such as proximal middle cerebral artery occlusion, may partially belong to the spectrum of moyamoya disease,

Table 11.1 Clinical significance of *RNF213* in moyamoya disease and related diseases

(A) *RNF213*-related intracranial steno-occlusive disease
1. Moyamoya disease
2. Unilateral moyamoya disease
3. Quasi-moyamoya disease
4. Non-moyamoya intracranial steno-occlusive disease (partly)
(B) Application of genetic testing by *RNF213* p.R4810K
1. Estimation of disease severity and progression
2. Family member screening to identify individuals who need periodical MRI examinations
3. Risk assessment of intracranial steno-occlusive disease in general population
(C) Unsolved issues in moyamoya spectrum
1. Other risk factors than *RNF213* are not known
2. Epigenetic modification in moyamoya spectrum is not well understood
3. Prospective study of genetic testing of *RNF213* p.R4810K is not performed
4. Efficacy of surgery for p.R4810K positive non-moyamoya patients is not tested

although it is difficult to distinguish such phenotypes from atherosclerotic disease. Bang et al. showed that a substantial proportion of Korean patients with intracranial steno-occlusive disease who did not fulfill the three diagnostic criteria for moyamoya disease (terminal ICA involvement, basal moyamoya, and bilateral involvement) had the p.R4810K variant. The variant was found in patients who met all three moyamoya criteria (75.6%), in those who met two criteria (57.5%), in those who met one criterion (28.6%), and also in those who met no criteria (20.0%), supporting the hypothesis that part of intracranial steno-occlusive disease without moyamoya vessels is associated with *RNF213* [20]. The presence of basal collaterals and bilateral involvement on angiography were independently associated with the presence of the p.R4810K variant. Negative remodeling of the terminal ICA and proximal middle cerebral artery is postulated as a marker to distinguish the moyamoya phenotype and the atherosclerotic phenotype [21–23]. Intracranial steno-occlusive disease is a complex disease that involves many factors. Further research on *RNF213* will improve our understanding of the entire disease.

11.3.3 Quasi-moyamoya Disease

Quasi-moyamoya disease is an angiographic moyamoya disease equivalent that is accompanied by known underlying diseases. In Japan, quasi-moyamoya disease constitutes 5.4% of all moyamoya cases [24]. Morimoto et al. showed that the p.R4810K variant was also associated with quasi-moyamoya disease [25]. They found this variant in 12 of 18 quasi-moyamoya disease patients. The frequency of p.R4810K carriers was significantly higher in patients with quasi-moyamoya

Table 11.2 The *RNF213* p.R4810K variant was observed in quasi-moyamoya disease patients with various comorbidities

ID	Age at onset (years)	Sex	Affected hemisphere	Comorbidity	Symptoms at onset	Genotype
1	11	F	B	NF1	TIA	Heterozygotes
2	4	F	U	NF1	Seizure	Wild type
3	9	M	U	NF1	Higher brain dysfunction	Wild type
4	35	F	B	NF1	IS	Wild type
5	17	M	B	NF1	IS	Wild type
6	41	F	B	Noonan syndrome	IS	Heterozygotes
7	26	F	B	Hyperthyroidism	TIA	Heterozygotes
8	28	F	B	Hyperthyroidism	TIA	Heterozygotes
9	59	F	U	Hyperthyroidism	IS	Heterozygotes
10	24	M	B	Hyperthyroidism	IS	Wild type
11	38	F	B	Hyperthyroidism	IS	Wild type
12	61	F	U	RA	Headache	Heterozygotes
13	70	F	B	RA	ICH	Heterozygotes
14	58	M	B	RA	Headache	Wild type
15	31	F	B	Down syndrome	ICH	Heterozygotes
16	15	M	B	Down syndrome	TIA	Wild type
17	5	F	B	Kawasaki disease	TIA	Heterozygotes
18	33	M	B	Kawasaki disease	TIA	Heterozygotes
19	57	F	B	SLE	ICH	Heterozygotes
20	61	F	B	Autoimmune pancreatitis	IS	Heterozygotes
21	69	F	B	Sjögren's syndrome Hashimoto's thyroiditis	ICH	Heterozygotes

F represents female, *M* male, *B* bilateral, *U* unilateral, *NF1* neurofibromatosis type 1, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *TIA* transient ischemic attack, *IS* ischemic stroke, *ICH* intracranial hemorrhage

disease than in controls (66.7% vs. 2.2%, odds ratio 89.0). The pR4810K variant was found in all types of comorbidities, although the frequency was different for each comorbidity (Table 11.2). The risk variant was found in 1 of 4 (25%) of patients with neurofibromatosis type 1 (NF1), 2/4 (50%) of patients with hyperthyroidism, 1 of 2 (50%) of patients with Down syndrome, and 100% of patients with other disorders such as Kawasaki disease (n=3), rheumatoid arthritis (n=2), or Noonan syndrome (n=1). Miyawaki et al., however, showed no association between p.R4810K and quasi-moyamoya disease [26], but this may be because of the different comorbidities such as irradiation (n=3) and central nervous system infection (n=2) and because of the small number in each population.

Clinical features of quasi-moyamoya disease are similar to that of moyamoya disease. Both of them show female predominance, familial occurrence (7%), and

two peak pattern of age distribution with higher peak at the first decade of life [27]. Given that revascularization surgery was also effective for quasi-moyamoya disease [28], a positive association of *RNF213* with quasi-moyamoya disease suggests that moyamoya disease and quasi-moyamoya disease may have common pathological background. Comorbidities such as Down syndrome or NF1 might increase the penetrance of the p.R4810K variant and increase the likelihood of having the moyamoya phenotype [25]. The proportion of unilateral cases is larger in quasi-moyamoya disease than moyamoya disease [27]. This may be due to smaller proportion of patients with the p.R4810K variant in quasi-moyamoya disease since the variant is associated with higher chances of bilateral involvement [6].

11.4 Clinical Application of p.R4810K Genotyping

There are three main possible applications of p.R4810K clinical testing, which include (1) estimation of disease severity and progression in patients with moyamoya disease, (2) screening of family members of patients with moyamoya disease to identify individuals who need periodic magnetic resonance imaging (MRI) screening examination, and (3) risk assessment of intracranial steno-occlusive disease in the general population (Table 11.1B).

11.4.1 Estimation of Disease Progression: Age at Onset and Disease Severity

The homozygous (AA genotype) p.R4810K variant was reported to be associated with an earlier age at onset compared with the heterozygous (GA genotype) variant and the wild-type (GG genotype) control [5, 6]. Most patients with the AA genotype develop moyamoya disease before 4 years of age. Homozygosity was also associated with involvement of bilateral hemispheres and posterior circulation, which might increase the severity of the disease [5, 6, 29]. Therefore, genetic testing to find patients homozygous for p.R4810K could be used to predict disease severity and progression (Fig. 11.3). Given that most patients with homozygotes develop moyamoya disease by 4 years of age, genetic testing would be necessary at their infancy, and genetic counseling should adequately be provided. Involvement of posterior circulation is associated with poor clinical outcome [30], and therefore revascularization surgery in an earlier stage may be considered. Importantly, some patients with homozygotes develop moyamoya disease between 50 and 60 years of age, and some are not affected by moyamoya disease. Additionally, the proportion of the AA genotype is low (<10%) [1, 5], and thus, clinical benefit of this genetic testing to predict disease severity may be limited. On the other hand, there were no

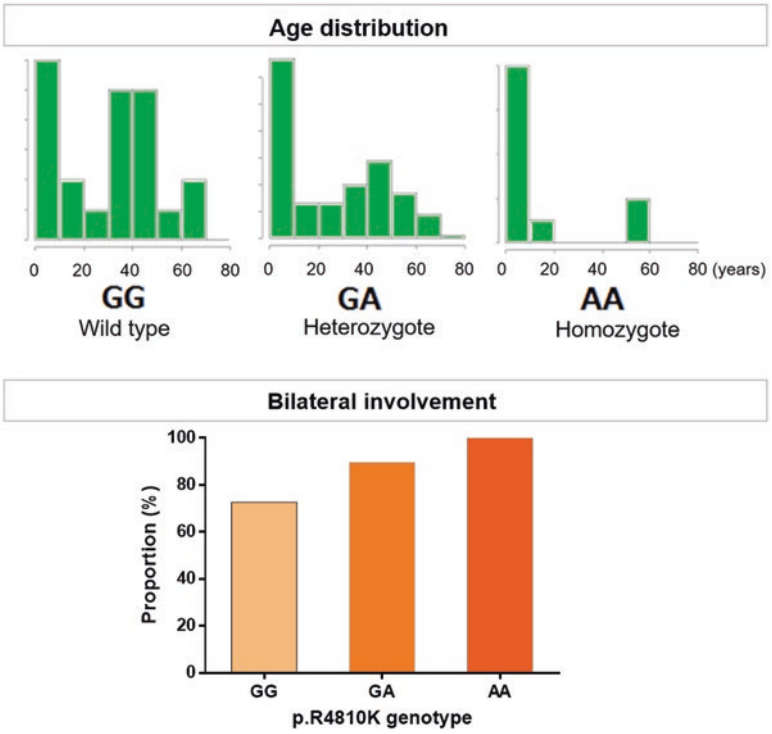
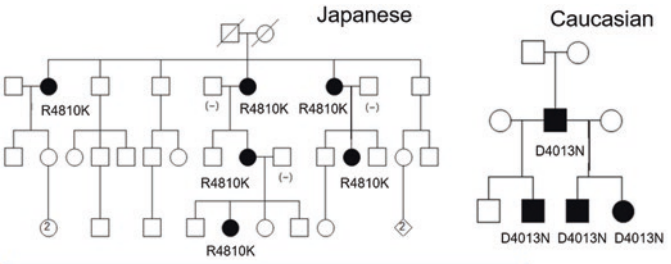


Fig. 11.3 Difference in age distribution and the proportion of bilateral involvement among the genotypes of *RNF213* p.R4810K in patients with moyamoya disease. Homozygotes (two alleles of p.R4810K variant) were associated with an earlier age at onset, and most patients developed moyamoya disease by the age of 4 years. Gene dose effect of p.R4810K was observed for the proportion of bilateral involvement. Genotyping of the p.R4810K variant may be useful to estimate the severity and the risk of progression of moyamoya disease

significant differences in clinical features between patients with the heterozygous variant and patients with the wild type, although bilateral involvement seems to be higher in patients with the heterozygous variant.

11.4.2 Risk Assessment of Cerebrovascular Disease for Family Members of Moyamoya Disease Patients

The proportion of familial moyamoya disease has been reported to be 10.0–15.4% [17, 19]. This percentage has been increasing, probably because of an increasing number of asymptomatic patients who have been diagnosed using MRI. In fact, after the screening examination by transcranial Doppler sonography followed by MR angiography for family members of patients with moyamoya disease, the proportion of familial moyamoya disease patients increased from 7 to 15% in the



Area	R4810K	Other mutations
Japan	90% (145/161) [42/42 families]	0% (0/161)
Korea	79% (30/38) [1 /1 family]	0% (0/38)
China	23% (12/52)	14% (7/52)
Europe	0% (0/50)	8% (4/50) [1/1 family]

Data from Liu et al. PLoS One 2011

Fig. 11.4 Contribution of *RNF213* in moyamoya disease in different ethnic populations. According to the data of published article by Liu et al., *RNF213* mutations were observed in all of the familial cases of familial moyamoya disease. The p.R4810K variant was observed in the Japanese and Korean families, and the p.D4013N variant was observed in the Caucasian family. In the Chinese population, the proportion of p.R4810K was lower than that in the Japanese or Korean populations, but other mutations were detected in 14% of cases. In Europe, p.R4810K was not observed in either patients with moyamoya disease or in the general population. Other mutations were observed in 8% of the patients. These data showed that significance of *RNF213* and its main susceptibility variant, p.R4810K, was different among the populations

Chinese population [31]. Genetic testing for p.R4810K in family members of moyamoya disease patients is expected to be effective to find individuals who may benefit from periodic MRI screening examinations; however, it has not been proved yet.

In Japan, p.R4810K was detected in 95–100% of patients with familial moyamoya disease, and other mutations were infrequent (Fig. 11.4) [1]. Therefore, p.R4810K genotyping, but not sequencing, would be sufficient in most cases to estimate the risk of developing intracranial steno-occlusive disease. However, in Western countries, p.R4810K was not polymorphic, and no familial moyamoya disease related to p.R4810K has been reported. D4013N was found in Caucasian families with familial moyamoya disease (Fig. 11.4) [1, 10]. Although genetic testing of D4013N cannot be performed for all family members of patients with sporadic moyamoya disease, it may be useful for family members of patients with familial moyamoya disease where two or more people are affected. In China, p.A4399T is another common variant found in 16.5% of patients, whereas p.R4810K was found in 13.1–31.4% [1, 7, 32]. The p.A4399T variant was shown to be associated with the hemorrhagic presentation of moyamoya disease in the Chinese population [7]. The benefits of p.A4399T genotyping in family members of Chinese patients with moyamoya disease need to be investigated.

11.4.3 Significance of p.R4810K for Risk Assessment in the General Population

Recently, direct-to-consumer (DTC) genetic testing, which is commercially based genetic testing to assess the risk of various kinds of diseases, has become widely available. Clinicians who do not specialize in genetics still have some genetic knowledge. In 2014, DTC genetic testing to assess the risk of moyamoya disease began, but rs6565681 instead of rs112735431 (p.R4810K) was used. The variant rs6565681 is observed in 36.7% of the general population, and the odds ratio of having moyamoya disease was only approximately 4.8 [2]. Considering that moyamoya disease is a rare disease, even if a person has the rs6565681 variant, there is no substantial risk of developing moyamoya disease (the risk of developing moyamoya disease is about 1 in 10,000 individuals for those who have rs6565681). Thus, a more specific strategy is needed for the genetic screening test for moyamoya disease in those without a family history of this disease.

The usefulness of p.R4810K genotyping for the risk assessment of intracranial steno-occlusive disease in the general population remains unknown. A prospective cohort study assessing the natural history of asymptomatic patients with moyamoya disease is ongoing (AMORE study). Additionally, the natural history of patients with *RNF213*-related vascular disease needs to be investigated in future studies.

11.5 Future Prospective

Although identification of a susceptibility gene *RNF213* revealed many aspects of moyamoya spectrum disease, there remains a lot of issues to be solved for better diagnosis and treatment for the disease (Table 11.1C).

11.5.1 Environmental Risk Factors and Associated Genetic Factors

Because penetrance of the p.R4810K variant is low, other factors should be involved in the pathogenesis of moyamoya disease and other related steno-occlusive diseases. Interaction of *RNF213* with diabetes and hypertension was shown in both animal and human studies. The p.R4810K variant was inversely associated with diabetes [20], and it was positively associated with higher systolic blood pressure [33]. Interaction of the *RNF213* variants with smoking or other atherosclerotic risk factors remains undetermined. Elucidation of modifiable risk factors that affect *RNF213* p.R4810K function may lead to the development of new treatment for intracranial steno-occlusive disease including moyamoya disease.

11.5.2 Epigenetic Modification

The difference in the prevalence between men and women, discordant phenotypes between identical twin pairs, and different clinical characteristics between paternal and maternal transmission suggest that epigenetic modification is involved in the pathogenesis of moyamoya disease [14]. Therefore, tissue-specific methylation analysis may provide important information. If epigenetic mechanisms are associated with a reduced risk of moyamoya disease, the mechanism may be used to control the risk of this disease.

11.5.3 Development of Novel Biomarkers and New Treatment Options

In non-Asian populations, p.R4810K is not polymorphic, and the variant cannot be used for a genetic screening examination [1, 10]. If the main signaling pathway that is common to patients with moyamoya disease worldwide is determined by investigating *RNF213* and other susceptibility genes, it could lead to the development of a novel biomarker for moyamoya disease and related disorders.

As shown in the Japan Adult Moyamoya (JAM) trial and other studies [34, 35], revascularization surgery is effective to prevent stroke in patients with moyamoya disease. However, efficacy of revascularization surgery was not shown in patients with atherosclerotic cerebrovascular disease, as indicated by the Carotid Occlusion Surgery Study (COSS) [36]. However, the effectiveness of revascularization surgery for patients with *RNF213*-related steno-occlusive disease remains unknown. The *RNF213* mutation may be a marker to identify patients with intracranial steno-occlusive diseases who will benefit from revascularization surgery.

11.6 Conclusions

RNF213 is a key molecule to better understand the pathogenesis of moyamoya disease and its related steno-occlusive disease, although identification of other cofactors and their relationship with *RNF213* is also required. Genetic testing by the p.R4810K has an impact on clinical practice of intracranial steno-occlusive disease including moyamoya disease in East Asian populations. Further research on *RNF213* will show the difference between moyamoya disease and atherosclerotic steno-occlusive disease.

References

1. Liu W et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542.
2. Kamada F et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40.
3. Mineharu Y et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70(24 Pt 2):2357–63.
4. Miyawaki S et al. Genetic variant RNF213 c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2013;44(10):2894–7.
5. Miyatake S et al. Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78(11):803–10.
6. Moteki Y et al. Systematic validation of RNF213 coding variants in Japanese patients with Moyamoya disease. J Am Heart Assoc. 2015;4(5):e001862.
7. Wu Z et al. Molecular analysis of RNF213 gene for moyamoya disease in the Chinese Han population. PLoS One. 2012;7(10):e48179.
8. Wang X et al. Impacts and interactions of PDGFRB, MMP-3, TIMP-2, and RNF213 polymorphisms on the risk of Moyamoya disease in Han Chinese human subjects. Gene. 2013;526(2):437–42.
9. Jang MA et al. Frequency of the moyamoya-related RNF213 p.Arg4810Lys variant in 1,516 Korean individuals. BMC Med Genet. 2015;16:109.
10. Cecchi AC et al. RNF213 rare variants in an ethnically diverse population with Moyamoya disease. Stroke. 2014;45(11):3200–7.
11. Shoemaker LD et al. Disease variant landscape of a large multiethnic population of Moyamoya patients by exome sequencing. G3 (Bethesda). 2016;6(1):41–9.
12. Ma J et al. RNF213 polymorphism and Moyamoya disease: a systematic review and meta-analysis. Neurol India. 2013;61(1):35–9.
13. Sun XS et al. The association between the ring finger protein 213 (RNF213) polymorphisms and moyamoya disease susceptibility: a meta-analysis based on case-control studies. Mol Gen Genomics. 2016;291(3):1193–203.
14. Mineharu Y et al. Inheritance pattern of familial moyamoya disease: autosomal dominant mode and genomic imprinting. J Neurol Neurosurg Psychiatry. 2006;77(9):1025–9.
15. Liu W et al. Distribution of moyamoya disease susceptibility polymorphism p.R4810K in RNF213 in East and Southeast Asian populations. Neurol Med Chir (Tokyo). 2012;52(5):299–303.
16. Miyawaki S et al. Identification of a genetic variant common to moyamoya disease and intracranial major artery stenosis/occlusion. Stroke. 2012;43(12):3371–4.
17. Wakai K et al. Epidemiological features of moyamoya disease in Japan: findings from a nationwide survey. Clin Neurol Neurosurg. 1997;99(Suppl 2):S1–5.
18. Hoshino H et al. Epidemiological features of moyamoya disease in Japan. Neurol Med Chir (Tokyo). 2012;52(5):295–8.
19. Baba T, Houkin K, Kuroda S. Novel epidemiological features of moyamoya disease. J Neurol Neurosurg Psychiatry. 2008;79(8):900–4.
20. Bang OY et al. Adult Moyamoya disease: a burden of intracranial stenosis in East Asians? PLoS One. 2015;10(6):e0130663.
21. Kuroda S et al. Specific shrinkage of carotid forks in Moyamoya disease: a novel key finding for diagnosis. Neurol Med Chir (Tokyo). 2015;55(10):796–804.
22. Kaku Y et al. Outer-diameter narrowing of the internal carotid and middle cerebral arteries in moyamoya disease detected on 3D constructive interference in steady-state MR image: is arterial constrictive remodeling a major pathogenesis? Acta Neurochir. 2012;154(12):2151–7.
23. Yuan M et al. High-resolution MR imaging of the arterial wall in moyamoya disease. Neurosci Lett. 2015;584:77–82.

24. Hayashi K et al. An epidemiological survey of moyamoya disease, unilateral moyamoya disease and quasi-moyamoya disease in Japan. Clin Neurol Neurosurg. 2013;115(7):930–3.
25. Morimoto T et al. Significant association of the RNF213 p.R4810K polymorphism with quasi-moyamoya disease. J Stroke Cerebrovasc Dis. 2016;25:2632–6.
26. Miyawaki S et al. Genetic analysis of RNF213 c.14576G>A variant in nonatherosclerotic quasi-moyamoya disease. J Stroke Cerebrovasc Dis. 2015;24(5):1075–9.
27. Hayashi K et al. Nationwide survey on quasi-moyamoya disease in Japan. Acta Neurochir. 2014;156(5):935–40.
28. Jea A et al. Moyamoya syndrome associated with Down syndrome: outcome after surgical revascularization. Pediatrics. 2005;116(5):e694–701.
29. Kim EH et al. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg. 2016;124(5):1221–7.
30. Funaki T et al. Impact of posterior cerebral artery involvement on long-term clinical and social outcome of pediatric moyamoya disease. J Neurosurg Pediatr. 2013;12(6):626–32.
31. He Y et al. Wavelet analysis of cerebral oxygenation oscillations in the screening of Moyamoya disease. Biomed Mater Eng. 2014;24(6):3463–9.
32. Zhang, Q., et al. RNF213 as the major susceptibility gene for Chinese patients with moyamoya disease and its clinical relevance. J Neurosurg. 2016; 1–8.
33. Koizumi A et al. P.R4810K, a polymorphism of RNF213, the susceptibility gene for moyamoya disease, is associated with blood pressure. Environ Health Prev Med. 2013;18(2):121–9.
34. Miyamoto S et al. Effects of extracranial-intracranial bypass for patients with hemorrhagic moyamoya disease: results of the Japan Adult Moyamoya Trial. Stroke. 2014;45(5):1415–21.
35. Takahashi JC et al. Significance of the hemorrhagic site for recurrent bleeding: prespecified analysis in the Japan Adult Moyamoya Trial. Stroke. 2016;47(1):37–43.
36. Powers WJ et al. Extracranial-intracranial bypass surgery for stroke prevention in hemodynamic cerebral ischemia: the Carotid Occlusion Surgery Study randomized trial. JAMA. 2011;306(18):1983–92.

Chapter 12
Significance of *RNF213* in Clinical Management in China

Zheng-Shan Zhang and Lian Duan

Abstract The ring finger protein 213 gene (*RNF213*) is the most important susceptibility gene for moyamoya disease (MMD). The relationship between the *RNF213* polymorphisms and MMD risk has also been identified in Chinese population. As the most common polymorphism in East Asian population, the p.R4810K (c.14576G>A) variant in *RNF213* has been considered a founder mutation; however, the prevalence of p.R4810K variant is different between Japanese, Korean, and Chinese. In addition, several novel variants are identified only in Chinese patients but not in Korean and Japanese patients. These results suggest that the mutational spectrum of *RNF213* gene may be different among different populations. MMD in Chinese population probably has more complex genetic background compared to Japanese and Korean MMD. We review the recent progress and significance of *RNF213* in clinical management in Chinese MMD patients in this chapter.

Keywords *RNF213* • China • Clinical management

12.1 Introduction

The ring finger protein 213 gene (*RNF213*) is the only susceptibility gene for moyamoya disease (MMD) identified by both genome-wide association studies and exon sequencing. Several studies have been conducted to identify the relationship between the *RNF213* polymorphisms and MMD risk [1–5]. These studies demonstrate that p.R4810K (c.14576G>A) variant is a founder mutation identified commonly in East Asian population; however, the prevalence of p.R4810K variant is different between Japanese, Korean, and Chinese [6–8]. One research found that the p.R4810Kvariant is found in 90% of Japanese, 79% of Korean, and 23% of Chinese MMD patients. The presence of this variant strongly increases the risk of

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developing MMD with an odds ratio (OR) of 338.9 ($p=10^{-100}$) in Japanese, 135.6 ($p=10^{-25}$) in Korean, and 14.7 ($p=10^{-4}$) in Chinese populations. In addition, several novel variants are identified only in Chinese patients but not in Korean and Japanese patients [2]. We hypothesize that MMD in Chinese population has more complex genetic background compared to Japanese and Korean MMD.

The study of *RNF213* and MMD in Chinese is still at early stage currently, and there are only a small number of reports in this area (Table 12.1). We will review the recent progress and significance of *RNF213* in clinical management in Chinese MMD patients.

12.2 Significance of Mutations in *RNF213* in Chinese Population

12.2.1 p.R4810K Mutation

The p.R4810K mutation in *RNF213* is the most common and important single nucleotide polymorphism found in Chinese MMD patients. A strong association between the p.R4810K polymorphism and MMD in Chinese patients is first demonstrated by a research group in Japan in 2011 [2]. Several studies in China have been performed to focus on the p.R4810K polymorphism susceptibility in Chinese MMD patients [9–15] (Table 12.2). Among Chinese Han ethnic group with MMD, the prevalence of p.R4810K mutant in sporadic MMD ranges from 9.3% to 10.9%, and the prevalence reaches 80% in familial MMD. However, the prevalence of p.R4810K mutant in control group of Chinese Han population (without MMD) is only 0.39–1.03%. These studies confirm that the p.R4810K mutation is much more frequently found in Chinese MMD patients than in the general population, and p.R4810K mutation is associated with MMD susceptibility in Chinese and increases the risk for MMD. In addition, these results suggest that the risk of MMD is higher in the familiar MMD patients carrying AA and AG genotypes of p.R4810K than the sporadic patients, indicating that p.R4810K screening would be an appropriate approach to identify asymptomatic patients, especially those who have a family history of MMD. Screening of the p.R4810K variant should be performed in Chinese MMD patients, especially those patients with positive family history.

Moreover, the p.R4810K mutation may be related to the subtype of MMD in Chinese Hans. One study found that the frequency of the mutant A allele is significantly higher in the ischemic group than in the hemorrhagic group [9]. This finding is consistent with another research demonstrating that p.R4810K homozygote patients present initially with infarction more often than p.R4810K heterozygotes and wild types [5]. These results further suggest that clinical manifestations of MMD may be associated with genetic background.

The association between the p.R4810K mutant and the genetic susceptibility of MMD in other Chinese ethnic groups such as Taiwanese population and Guangxi

Table 12.1 *RNF213* mutations associated with MMD patients in Chinese

Mutations	Patients' background	Cases/ controls	Main findings	References
p.R4810K	Chinese	52/150	p.R4810K polymorphism increases risk of MMD	[2]
	Han nationality	170/507	p.R4810K polymorphism increases risk of MMD and the significant association between p.R4810K and ischemia MMD	[9]
	Han nationality	64/96	p.R4810K polymorphism increases risk of MMD	[11]
	Han nationality	96/96	p.R4810K polymorphism increases risk of MMD	[10]
	Zhuang nationality	52/80	p.R4810K polymorphism increases risk of MMD	[12]
	Taiwanese	36/500	p.R4810K mutation is the most common mutation among the *RNF213* mutations (54.5%), and the incidence of the p.R4810K mutation in Taiwan is 16.7 % (6/36). The prevalence of this mutation among the general population of Taiwan remains to be determined	[13]
p.A4399T	Han nationality	170/507	p.A4399T polymorphism increases risk of MMD and the significant association between p.A4399T and hemorrhage MMD	[9]
	Han nationality	96/96	p.A4399T polymorphism increases risk of MMD	[11]
	Zhuang nationality	52/80	No association between p.A4399T and hemorrhage MMD	[12]
p.A5021V	Chinese	52/150	p.A5021V mutation was found in 2 out of 52 Chinese MMD patients	[2]
	Han nationality	20/20	p.A5021V was detected in 1 out of 20 cases by gene sequencing	[9]
	Han nationality	96/96	No association between p.A5021V and MMD	[11]
	Zhuang nationality	52/80	No association between p.A5021V and MMD	[12]
p.E4950D	Chinese	52/150	p. E4950D mutation was found in 2 out of 52 Chinese MMD patients	[2]
	Han nationality	20/20	p. E4950D was detected in 1 out of 20 cases by gene sequencing	[9]
p.E5176G	Chinese	52/150	p.E5176G mutation was found in 1 out of 52 Chinese MMD patients	[2]
p.D5160E	Chinese	52/150	p.D5160E mutation was found in 1 out of 52 Chinese MMD patients	[2]

(continued)

Table 12.1 (continued)

Mutations	Patients' background	Cases/ controls	Main findings	References
p.D4863N	Chinese	52/150	p.D4863N mutation was found in 1 out of 52 Chinese MMD patients	[2]
p.T4586P	Han nationality	20/20	p.T4586P was detected in 1 out of 20 cases by gene sequencing	[9]
p.Q4367L	Han nationality	20/20	p.Q4367L was detected as a new mutation in 1 out of 20 cases by gene sequencing	[9]
p.P4007R	Han nationality	20/20	p.P4007R was detected as a new mutation in 1 out of 20 cases by gene sequencing	[9]
p.M5136I	Han nationality	20/20	p.M5136I was detected in 1 out of 20 cases by gene sequencing	[9]
p.L4631V	Han nationality	20/20	p.L4631V was detected as a new mutation in 1 out of 20 cases by gene sequencing	[9]
p.A1622V	Taiwanese	36/500	p.A1622V mutation was found in 1 out of 36 Taiwanese MMD patients	[13]
p.R4131C	Taiwanese	36/500	p.R4131C mutation was found in 2 out of 36 Taiwanese MMD patients	[13]
p.V3933M	Taiwanese	36/500	p.V3933M mutation was found in 2 out of 36 Taiwanese MMD patients	[13]

Zhuang ethnic group has been studied recently [12, 13]. It has been found that 30.6% of Taiwanese MMD patients carry an *RNF213* mutation, and the missense p.R4810K mutation is the most common mutation among the *RNF213* mutations in Taiwanese population. Although the prevalence of this mutation among the general population of Taiwanese remains to be determined, these data suggest that the p. R4810K mutation is probably associated with MMD in Taiwanese population. Another study performed in Guangxi Zhuang ethnic group also found that the genotype (GA+AA) frequencies of p.R4810K mutant in *RNF213* are significantly higher in MMD group than in the normal control group. Therefore, the p.R4810K mutation may be associated with MMD patients in both Chinese Hans and other ethnic groups.

Furthermore, studies conducted in Taiwanese population and Guangxi Zhuang ethnic group have found that the genotype (GA+AA) frequencies of p.R4810K mutant in both MMD group and non-MMD group with intracranial major artery stenosis/occlusion (ICASO) are significantly higher than in the normal control group [12, 13]. This finding is consistent with another research conducted in Japanese population [16]. Therefore, polymorphisms of p.R4810K in *RNF213* might be related to MMD and non-MMD patients with ICASO in Taiwanese and Guangxi Zhuang ethnic group.

Table 12.2 Distribution of p.R4810K mutation in Chinese MMD patients

References	Cases/controls	Subtype	MMD	Control
[2]	52/100	GG	40(76.9)	98(98.0)
		GA	11(21.1)	2(2.0)
		AA	1(2.0)	0(0)
		G	91(87.5)	198(99.0)
		A	13(12.5)	2(1.0)
[11]	64/96	GG	57(89.1)	95(99.0)
		GA	6(9.4)	1(1.0)
		AA	1(1.5)	0(0)
		G	120(93.0)	191(99.5)
		A	8(7.0)	1(0.5)
[10]	96/96	GG	87(90.6)	95(99.0)
		GA	8(8.4)	1(1.0)
		AA	1(1.0)	0(0)
		G	182(94.8)	191(99.5)
		A	10(5.2)	1(0.5)
[9]	170/507	GG	148(87.0)	505(99.6)
		GA	21(12.4)	2(0.4)
		AA	1(0.6)	0(0)
		G	317(93.2)	1012(99.8)
		A	23(6.8)	2(0.2)
[12]	52/80	GG	45(86.5)	79(98.8)
		GA	6(11.5)	1(1.2)
		AA	1(2.0)	0(0)
		G	96(92.3)	159(99.4)
		A	8(7.7)	1(0.6)
[13]	36/500	GG	30(83.3)	NA
		GA+AA	6(16.7)	NA
		G	NA	NA
		A	NA	NA

NA not applicable

12.2.2 p.A4399T Mutation

The p.A4399T mutation is considered a potential risk factor for MMD, especially the hemorrhagic subtype in Chinese population. Two studies conducted in Chinese Hans have suggested that the p.A4399T variation which is a common polymorphism in both disease and control groups also increases the risk for MMD [9, 10]. The frequency of G allele and G/G genotype of p.A4399T in sporadic MMD patients of Chinese Hans is higher than in controls (Table 12.3). In addition, there is a significant difference in both the *RNF213* p.A4399T genotype and allele frequency between the hemorrhagic and ischemic groups. Meanwhile, comparing with p.R4810K, p.A4399T is less frequent in familial cases, further confirming that the

Table 12.3 Distribution of p.A4399T and p.A5021V mutations in Chinese MMD patients

Mutations	References	Cases/controls	Subtype	MMD(%)	Control(%)
p.A4399T	[9]	170/507	GG	142(83.5)	462(91.1)
			GA	27(15.9)	45(8.9)
			AA	1(0.6)	0(0)
			G	311(91.5)	969(95.6)
			A	29(8.5)	45(4.4)
	[12]	52/80	GG	49(94.2)	80(100.0)
			GA	3(5.8)	0(0)
			AA	0(0)	0(0)
			G	101(97.1)	160(100.0)
			A	3(2.9)	0(0)
	[10]	96/96	GG	96(100.0)	85(88.5)
			GA	0(0)	11(11.5)
			AA	0(0)	0(0)
			G	192(100.0)	170(88.5)
			A	0(0)	22(11.5)
p.A5021V	[2]	52/100	CC	50(96.2)	100(100.0)
			CT	2(3.8)	0(0)
			TT	0(0)	0(0)
			C	102(98.1)	200(100.0)
			T	2(1.9)	0(0)
	[11]	50/90	CC	47(94.0)	89(98.9)
			CT	3(6.0)	1(1.1)
			TT	0(0)	0(0)
			C	97(97.0)	179(99.4)
			T	3(3.0)	1(0.6)
	[12]	52/80	CC	49(94.2)	78(97.5)
			CT	3(5.8)	2(2.5)
			TT	0(0)	0(0)
			C	101(97.1)	158(98.8)
			T	3(2.9)	2(1.2)

NA not applicable

p.R4810K mutation is the main genetic driver for MMD. Furthermore, different variations with no LD (D' = 0.496, $r2$ = 0.00) (p.R4810K and p.A4399T) are involved in pathogenic risks of different clinical manifestations (ischemia and hemorrhage) [9]. However, the allele and genotype frequencies of p.A4399T show no statistical significance between MMD and normal control group in Guangxi Zhuang population [12]. A meta-analysis of the association between the ring finger protein 213 (*RNF213*) polymorphisms and MMD susceptibility shows that p.A4399T is not related with MMD risk in any genetic model, neither in the Japanese population nor the Chinese population [15]. It is possible that the minor allele frequency (MAF) of p.A4399T is too low and the sample size is too small to show statistical significance.

12.2.3 p.A5021V Mutation

The p.A5021V mutation is a rare variant in *RNF213* which may be related to the Chinese MMD patients. It is first found in 2 out of 52Chinese MMD patients by Liu et al. in 2011, [2] and it is not found in non-MMD Chinese. Several studies later also detected p.A5021V mutation in some Chinese MMD patients and few controls [9, 11, 12]. But the allele and genotype frequencies of p.A5021V show no statistical significance between MMD group and normal control group. This may represent a random mutation because of the low frequency. However, a recent meta-analysis has found that the p.A5021V mutation is associated with MMD risk only in Chinese Han population, but not in other ethnicities [15]. More studies with larger sample size and better study design are needed to validate the association between p.A5021V mutation and MMD patients in Chinese population.

12.2.4 Other Mutations

Several other variants in *RNF213* have been identified in Chinese MMD patients. Among these variants, p.E4950D is detected in studies conducted by Liu et al. [2] and Wu et al. [9] Three other variants (p.D4863N, p.D5160E, and p.E5176G) are found only in Chinese MMD patients by Liu et al. in 2011 [2]. Five other variants (p.L4631V, p.Q4367L, p.P4007R, p.M5136I, and p.T4586P) are found by Wu et al. [9], and three mutations (p.A1622V, p.V3933M, p.R4131C) are found in MMD patients of Taiwanese [13]. However, the frequencies of all these variants are so low, and it is uncertain if there is a relationship between these variants and Chinese MMD patients.

12.3 Conclusions

In summary, these results suggest that the *RNF213* gene is associated with MMD in the Chinese population, and the mutational spectrum of *RNF213* gene is different among different populations. However, the sample size of cases and controls analyzed in these studies is small; studies with larger sample size and better design are needed to validate these results in the future. Secondly, no study has been done so far to analyze the relationship between *RNF213* gene polymorphisms and unilateral or bilateral MMD, age of onset, and other clinical aspects of MMD due to insufficient information. Well-designed studies should be conducted to confirm the role of *RNF213* and other susceptibility genes in pathogenesis and pathophysiology of MMD.

12.4 Future Prospects

Moyamoya disease is a progressive disease in most patients and can lead to devastating neurologic deficits and intellectual impairments if untreated. Early diagnosis and surgical intervention are very important. However, there is no biomarker for early diagnosis of MMD so far. In addition, the current diagnosis of MMD is based on the angiographic characteristics, and there are some unanswered questions, including (1) the relationship and differences between MMD and moyamoya syndrome, (2) whether bilateral and unilateral MMD belong to a single entity, and (3) how many subgroups MMD should divide into. Now, the genetic progress of RNF123 in MMD may provide us a tool to resolve these problems. In the future, *RNF213* may be applied as the biomarker of MMD to illustrate the entire spectrum of MMD and delineate the natural course of carriers of *RNF213* mutations in epidemiology.

With the increasing number of patients diagnosed with MMD in China, some MMD clinical centers have been formed throughout the country in the past 10 years, such as the Neurosurgery Department of PLA 307 hospital. Large number of specimens and clinical data of Chinese MMD patients are collected and archived in these institutions. We will conduct more multicenter, large sample, and comprehensive researches to combine clinical information, genetics, and other fields such as autoimmunity and endothelial progenitor cells to further understand the etiology and pathogenesis of MMD.

References

1. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, Kanno J, Niihori T, Ono M, Ishii N, Owada Y, Fujimura M, Mashimo Y, Suzuki Y, Hata A, Tsuchiya S, Tominaga T, Matsubara Y, Kure S. A genome-wide association study identifies *RNF213* as the first Moyamoya disease gene. J Hum Genet. 2011;56:34–40.
2. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, Hashikata H, Matsuura N, Yamazaki S, Toyoda A, Kikuta K, Takagi Y, Harada KH, Fujiyama A, Herzig R, Krischek B, Zou LP, Kim JE, Kitakaze M, Miyamoto S, Nagata K, Hashimoto N, Koizumi A. Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6:e22542.
3. 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. 2016;124:1221–7.
4. Jang MA, Shin S, Yoon JH, Ki CS. Frequency of the moyamoya-related *RNF213* p.Arg4810Lys variant in 1, 516 Korean individuals. BMC Med Genet. 2015;16:1–3.
5. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, Tsurusaki Y, Doi H, Sakai H, Saitsu H, Shimojima K, Yamamoto T, Higurashi M, Kawahara N, Kawauchi H, Nagasaka K, Okamoto N, Mori T, Koyano S, Kuroiwa Y, Taguri M, Morita S, Matsubara Y, Kure S, Matsumoto N. Homozygous c.14576G>A variant of *RNF213* predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78:803–10.

6. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of moyamoya disease and associated health risks explored through *RNF213*. Environ Health Prev Med. 2016;21:55–70.
7. Bersano A, Guey S, Bedini G, Nava S, Hervé D, Vajkoczy P, Tatlisumak T, Sareela M, van der Zwan A, Klijn CJ, Braun KP, Kronenburg A, Acerbi F, Brown MM, Calviere L, Cordonnier C, Henon H, Thines L, Khan N, Czabanka M, Kraemer M, Simister R, Prontera P, Tournier-Lasserve E, Parati E. Research progresses in understanding the pathophysiology of Moyamoya disease. Cerebrovasc Dis. 2016;41:105–18.
8. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, Tominaga T. Genetics and biomarkers of Moyamoya disease: significance of *RNF213* as a susceptibility gene. J Stroke. 2014;16:65–72.
9. Wu Z, Jiang H, Zhang L, Xu X, Zhang X, Kang Z, Song DL, Zhang J, Guan M, Gu YX. Molecular analysis of *RNF213* gene for Moyamoya disease in the Chinese Han population. PLoS One. 2012;7:e48179.
10. Wang X, Zhang Z, Liu W, Xiong Y, Sun W, Huang X, Jiang Y, Ni G, Sun W, Zhou L, Wu L, Zhu W, Li H, Liu X, Xu G. Impacts and interactions of *PDGFRB*, *MMP-3*, *TIMP-2*, and *RNF213* polymorphisms on the risk of Moyamoya disease in Han Chinese human subjects. Gene. 2013;526:437–42.
11. Wang X, Huang X, Zhang Z, Liu W, Zhang M, Sun W, Ni G, Sun W, Cai Q, Zhou L, Liu X, Xu G. Association between ring finger protein 213 gene polymorphisms and the genetic susceptibility of adult moyamoya disease in a Han Chinese population. Chin J Neurol. 2013;46:229–32.
12. Huang YL, Cheng DB, Zhang JD, Xiao YS, Luo M. Association between *RNF213* gene polymorphisms and the genetic susceptibility of adult Moyamoya disease of Zhuang population in Guangxi. J Apoplexy Nerv Dis. 2015;32:918–21.
13. Lee MJ, Chen YF, Fan PC, Wang KC, Wang K, Wang J, Kuo MF. Mutation genotypes of *RNF213* gene from moyamoya patients in Taiwan. J Neurol Sci. 2015;353:161–5.
14. Ma J, Liu Y, Ma L, Huang S, Li H, You C. *RNF213* polymorphism and Moyamoya disease: a systematic review and meta-analysis. Neurol India. 2013;61:35–9.
15. Sun XS, Wen J, Li JX, Lai R, Wang YF, Liu HJ, Sheng WL. The association between the ring finger protein 213 (*RNF213*) polymorphisms and moyamoya disease susceptibility: a meta-analysis based on case-control studies. Mol Gen Genomics. 2016;291:1193–203.
16. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, Nakatomi H, Shimizu T, Saito N. Genetic variant *RNF213* c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2013;44:2894–7.

Chapter 13
The Different Forms of Moyamoya Disease and Their Clinical Management

Niklas von Spreckelsen and Boris Krischek

Abstract Ever since the first description of moyamoya in 1957, many advances in diagnosis and treatment have been made. While the treatment of the disease mainly remains surgical due to lack of medical treatment options, the general clinical management as well as pre-, peri-, and postoperative care is of utmost importance in order to minimize the ramifications of the disease. In this chapter we try to depict the characteristics of different forms of moyamoya; discuss regional differences; give an overview of current methods in diagnosis, conservative, as well as surgical treatment; and illustrate crucial steps in the clinical management of moyamoya.

Keywords Clinical management • Regional differences • Moyamoya syndrome • Moyamoya disease

13.1 Disease and Syndrome

Patients harboring the "puff of smoke phenomenon" first described as a hypoplasia of the internal carotid artery [ICA] and its compensatory collateral vessels in 1957 [1] and later specified as a discrete pathology denominated "moyamoya" [2], per definition, either have the moyamoya disease or a moyamoya syndrome.

A variety of underlying factors, including genetic predispositions such as *RNF213* [3], are suspected to lead to the occlusion of the ICA; however, a common origin is yet to be discovered, and therefore the diagnosis of both, the syndrome and the disease, relies on the imaging of cerebral vessels by MRI and digital subtraction angiography.

Only if a patient has bilateral stenosis of the ICA and no associated systemic syndrome or condition, a "moyamoya disease" [MMD] can be diagnosed. In other cases where there is only a unilateral ICA stenosis present or there is an associated

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Table 13.1 Associated syndromes/diseases with moyamoya syndrome [5]

Fairly common	
Sickle cell disease	
Neurofibromatosis type 1	
Cranial therapeutic irradiation	
Down's syndrome	
Rare (single or few case reports)	Reported cases include
Infectious diseases	Meningitis, HIV, leptospirosis
Hematologic conditions other than sickle cell disease	Beta thalassemia, Fanconi anemia, hereditary spherocytosis
Vasculitis and autoimmune diseases	Systemic lupus erythematosus, diabetes mellitus type 1, Graves' disease and thyroiditis
Renal artery stenosis	
Congenital cardiac anomaly	

Due to the wide variety of cases connected to moyamoya, only part of the reported cases can be displayed here

disease or syndrome linked to moyamoya in addition to the ICA stenosis (bilateral or unilateral) (Table 13.1), it is called "moyamoya syndrome" [MMS]. Furthermore, patients with either MMD or MMS can be divided into an adult and a pediatric cohort. The diagnosis following the onset of symptoms spikes at the age of 5–10 years and in the fourth decade [4].

13.2 Regional Differences

Moyamoya is most frequently diagnosed in Japan; however, over the past years, an increasing number of cases have been reported all over the world, and with these, certain differences in clinical onset and disease progression have been reported. According to recent data, one can assume that not only a regional but also an ethnic aspect is a variable influencing certain characteristics of the disease. The incidence of moyamoya differs heavily within certain regions. The European form of moyamoya disease clearly differs from the Asian form. Clinically the timing of vasculopathy onset differs, and there is a lower rate of hemorrhage as compared to the Asian moyamoya disease. Single-nucleotide polymorphisms that play a role in atherosclerosis, vascular growth, and transformation processes have been found to be associated with the European form. Candidate gene associations found in Asian patients could not be replicated in European patients [6].

While the incidence in Japan is said to be 0.34–0.94/100,000, in North America it is estimated to be 0.086/100,000 [7]. However, a strong genetic predisposition seems to be at hand, since there was a much higher incidence of moyamoya in people with an Asian background living in California and Hawaii as compared to the Caucasian population [7]. Acker et al. were able to demonstrate similarities between European and North American moyamoya patients and concluded that a

Table 13.2 Clinical manifestations of moyamoya

	No. of patients (n=962)
TIA	353 (37%)
Frequent TIA	63 (7%)
Cerebral infarction	165 (17%)
Intracerebral hemorrhage	186 (19%)
Headache	57 (6%)
Epilepsy	29 (3%)
Asymptomatic	32 (3%)
Others	13 (1%)
Details unknown	64 (7%)

Hashimoto et al. (2012). Guidelines for diagnosis and treatment of Moyamoya disease (spontaneous occlusion of the circle of Willis) [15]

"MMD in a non-Asian ethnic background could be a distinct cerebrovascular disease entity" [8]. While the latest Japanese report shows the main peak of incidence in the pediatric cohort at 5–9 years and a secondary peak at around 40 years, the European cohort had a much later onset peak at the age of 40–49 and a secondary peak at the age of 11. In addition, there is a less frequent hemorrhage rate in the adult population in non-Asian patients. While 21–62% of patients in Asia present with intracranial hemorrhage, only 15% in North America and 8.5% in Europe become symptomatic through hemorrhage [7–10]. Also, unilateral moyamoya seems to be more common in non-Asian cohorts (10.6% of MMD patients in Japan vs. 17% in Europe and 17.8% in North America) [7–11].

13.3 Onset and Diagnosis

In general, symptoms experienced by moyamoya patients can be categorized in either ischemic symptoms, caused directly by the stenosis of the ICA such as transient ischemic attacks [TIAs], stroke, and seizures, or indirect symptoms caused by the collateralization, ranging from headaches, presumably caused by an increased blood flow in the meningeal arteries [12], to hemorrhagic events of vulnerable collateral vessels.

Whereas pediatric patients mostly present with ischemic symptoms, adult patients tend to become symptomatic due to hemorrhage [13, 14] (Table 13.2).

Because these symptoms caused by stroke, TIAs, or hemorrhage can occur due to a variety of reasons other than moyamoya, the diagnosis of MMD or MMS can only be made once MRI and/or DSA imaging is obtained. With the help of these angiographic images, the progress of the disease can be categorized in six different stages first introduced in 1969 by Suzuki [2] (Table 13.3). In addition to these stages and the DSA, other modalities prove useful in order to assess the individual progress

Table 13.3 Suzuki grading [2]

Suzuki and Takaku first described moyamoya and its staging in 1969
Stage I
"Narrowing of the carotid fork" [a]
Narrowed ICA bifurcation
Stage II
"Initiation of the moyamoya"
Dilated ACA, MCA, and narrowed ICA bifurcation with moyamoya change
Stage III
"Intensification of the moyamoya"
Further increase in moyamoya change of the ICA bifurcation and narrowed ACA and MCA
Stage IV
"Minimization of the moyamoya"
Moyamoya change reducing with occlusive changes in ICA and tenuous ACA and MCA
Stage V
"Reduction of the moyamoya"
Further decrease in moyamoya change with occlusion of ICA, ACA, and MCA
Stage VI
"Disappearance of the moyamoya"
ICA essentially disappeared with supply of the brain from ECA

[a]The description in inverted commas is that of Suzuki in the original paper

of MMD or MMS. Parenchymal damage as well as the degree of vascular stenosis can be visualized by MRI. A wide variety of functional imaging (SPECT, PET, fMRI) analyzing hemodynamics is available and can be used to further quantify the impairment of the cerebral blood flow [CBF] which has been shown to correlate with the incidence of stroke [16]. So far no standard imaging technique has been established to assess the hemodynamic status. All of the abovementioned modalities however can be helpful in treatment decisions and especially pre- and postoperative monitoring of the disease.

13.4 Screening

Due to the rarity of the disease, there are no recommendations for indiscriminate screening for moyamoya. However, there has been a recent study by Han et al. that might warrant the screening of first-degree relatives of MMD patients by noninvasive TCD [17, 18]. In patients with high-risk syndromes for MMS such as sickle cell disease, Down's syndrome, and neurofibromatosis type 1, special attention should be paid to possible ischemic-related symptoms, and imaging should be performed generously [19–22].

13.5 Medical Management

To date, there is no medical treatment available to halt or alter the progression of the ICA occlusion, and medical care in MMD or MMS mainly implies secondary prophylaxis of symptoms such as TIAs, stroke, and headaches.

Inhibitors of platelet aggregation such as acetylsalicylic acid (ASA) are used to prevent TIAs and strokes triggered by emboli due to microthrombi developed in areas of vascular stenosis [13, 23].

Calcium channel blockers are the second group of agents which help to manage some symptoms of MMD/MMS. Although the mechanism remains unclear, calcium channel blockers may alleviate headaches linked to moyamoya. Furthermore, they can mitigate or prevent TIAs by elevating the cerebral blood flow. With hypotension as a common side effect, calcium channel blockers have to be administered with great caution to avoid iatrogenic TIAs or stroke [19].

Only few studies have been performed looking explicitly at the natural history of MMS [24, 25]. However, in solely medically treated patients, Hallemeier found a risk for recurrence of stroke over the course of 5 years to be 65% in the same hemisphere if the hemodynamic reserve is derogated; in bilateral impairment, the risk was even higher with 82% over the same time frame [26].

In addition to the risk of recurrent stroke, a chronically reduced hemodynamic reserve can lead to impaired cognition and daily function most prominently in children, but also in adults [27–29].

Taking all of this into account, any symptomatic MMD or MMS should at least be considered for surgical intervention.

13.6 Surgical Management

Surgical revascularization in symptomatic MMD is increasingly accepted as the primary treatment. Several studies have shown the benefits of surgery compared to conservative medical treatment in short- as well as long-term follow-up [13, 26]. While the goal in surgery will always be to enhance the intracranial perfusion by connecting the intracranial to the extracranial blood supply, usually using the external carotid artery and therefore bypassing the ICA stenosis, there are two fundamentally different approaches which each have been adjusted and evolved over the past decades: the direct and the indirect bypass.

Direct techniques involve an immediate anastomosis of the extracranial vessel with the post-stenotic part of the ICA. Indirect procedures facilitate the growth of collateral vessels from extracranial tissue into the brain in watershed regions in danger of ischemic events.

Both approaches have been shown to lower the number of strokes and prevent further hemorrhage; however, so far there is no guideline for when to apply a direct or indirect technique. Historically, the direct approach has been used more frequently

in adults, while due to the small pediatric vessels and the greater surgical challenges, indirect approaches are often chosen in children. The majority of the literature comparing direct and indirect bypass has found no significant difference in long-term outcome between the two procedures [29–31], so each patient has to be assessed individually to determine the best approach. Several different techniques in direct anastomosis (e.g., STA to MCA bypass, STA to MCA/ACA bypass), indirect anastomosis (e.g., encephalodurosynangiosis, encephalomyosynangiosis, or encephaloduroarteriosynangiosis), or a combination of both have been described [31–34].

The perioperative management in both direct and indirect anastomoses however is similar in each approach and of utmost importance to avoid perioperative stroke and hemorrhage.

13.7 Perioperative and Acute Management

In all symptomatic patients, but especially in children, the threshold to a new ischemic event being induced due to temporary lowered blood pressure is very little. At the same time, such episodes are likely to be the cause of intermittent TIAs, strokes, and hemorrhages so that the acute treatment and perioperative management follow the same principles and aim to avoid:

- Hypotension
- Hypovolemia
- Hyperthermia
- Hypo- and hypercarbia

Hypovolemia and hyperthermia both can lead directly to hypotension and therefore lower the CBF. The autoregulated vasodilation of cerebral vessels caused by an increased pCO2 in diseased vessels compared to healthy arteries is minimal and by shifting the blood flow to dilated healthy vessels reduces the perfusion in affected areas [35]. Hypocapnia on the other hand leads to a general cerebral vasoconstriction and downregulates the overall CBF [35].

Therefore, special attention should be paid to an adequate pain management especially in children to avoid crying and concomitant hyperventilation. Furthermore, pre- and postoperative hydration with 1.25 or 1.5 of the normal maintenance rate as well as the administration of supplemental oxygen is suggested [13, 19, 35, 36].

Intraoperative management involves a rigorous monitoring and adjustment of blood pressure as well as extensive neurophysiological monitoring. Ideally, an arterial catheter should be placed before anesthesia induction. In children, however, the advantage of preinductional monitoring has to be weighed against the induced stress of placing the arterial catheter on the awake patient. Neurophysiological monitoring includes somatosensory-evoked potentials (SEPs), transcranial motor-evoked potentials (MEPs), and electroencephalography (EEG) and serves to detect any intraoperative changes in neurological function.

References

1. Takeuchi K, Shimizu K. Hypoplasia of the bilateral internal carotid arteries. Brain Nerve. 1957;9:37–43.
2. Suzuki J, Takaku A. Cerebrovascular moyamoya disease: disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20(3):288–99.
3. 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. In: Meisel A (ed) PLoS One. 2011;6(7):e22542. doi:10.1371/journal.pone.0022542.
4. Fukui M. Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis (Moyamoya disease). Clin Neurol Neurosurg. 1997;99:S238–40.
5. Roach ES, Golomb MR, Adams R, et al. Management of stroke in infants and children: a scientific statement from a special writing group of the american heart association stroke council and the council on cardiovascular disease in the young. Stroke. 2008;39(9):2644–91. doi:10.1161/STROKEAHA.108.189696.
6. Krischek B, Kasuya H, Khan N, Tatagiba M, Roder C, Kraemer M. Genetic and clinical characteristics of Moyamoya disease in Europeans. In:Trends in neurovascular surgery. Vienna: Springer; 2011. p. 31–4.
7. Kleinloog R, Regli L, Rinkel GJE, Klijn CJM. Regional differences in incidence and patient characteristics of moyamoya disease: a systematic review. J Neurol Neurosurg Psychiatry. 2012;83(5):531–6. doi:10.1136/jnnp-2011-301387.
8. Acker G, Goerdes S, Schneider UC, Schmiedek P, Czabanka M, Vajkoczy P. Distinct clinical and radiographic characteristics of moyamoya disease amongst European Caucasians. Eur J Neurol. 2015;22(6):1012–7. doi:10.1111/ene.12702.
9. Hoshino H, Izawa Y, Suzuki N. Epidemiological features of Moyamoya disease in Japan. Neurol Med Chir. 2012;52:295–8.
10. Uchino K, Johnston SC, Becker KJ, Tirschwell DL. Moyamoya disease in Washington State and California. Neurology. 2005;65(6):956–8. doi:10.1212/01.wnl.0000176066.33797.82.
11. Guzman R, Lee M, Achrol A, et al. Clinical outcome after 450 revascularization procedures for moyamoya disease: clinical article. J Neurosurg. 2009;111(5):927–35.
12. Seol HJ, Wang K-C, Kim S-K, Hwang Y-S, Kim KJ, Cho B-K. Headache in pediatric moyamoya disease: review of 204 consecutive cases. J Neurosurg Pediatr. 2005;103(5):439–42. doi:10.3171/ped.2005.103.5.0439.
13. Scott RM, Smith JL, Robertson RL, Madsen JR, Soriano SG, Rockoff MA. Long-term outcome in children with moyamoya syndrome after cranial revascularization by pial synangiosis. J Neurosurg. 2004;100(2 Suppl Pediatrics):142–9. doi:10.3171/ped.2004.100.2.0142.
14. Hee Han D, Kwon O-K, Jang Byun B, et al. A co-operative study: clinical characteristics of 334 Korean patients with Moyamoya disease treated at neurosurgical institutes (1976±1994).
15. Hashimoto N, Tominaga T, Miyamoto S, et al. Guidelines for diagnosis and treatment of moyamoya disease (spontaneous occlusion of the circle of Willis). Neurol Med Chir. 2012;52(5):245–66.
16. Lee M, Zaharchuk G, Guzman R, Achrol A, Bell-Stephens T, Steinberg GK. Quantitative hemodynamic studies in moyamoya disease: a review. Neurosurg Focus. 2009;26(4):E5. doi:10.3171/2009.1.FOCUS08300.
17. Han C, Feng H, Han YQ, et al. Prospective screening of family members with moyamoya disease patients. PLoS One. 2014;9(2):e88765. doi:10.1371/journal.pone.0088765.
18. Kim JS. Moyamoya disease: epidemiology, clinical features, and diagnosis. J Stroke. 2016;18(1):2–11. doi:10.5853/jos.2015.01627.
19. Scott RM, Smith ER. Moyamoya disease and Moyamoya syndrome. N Engl J Med. 2009;360(1):1226–37. doi:10.1056/NEJMra0804622.
20. Jea A, Smith ER, Robertson R, Scott RM. Moyamoya syndrome associated with Down syndrome: outcome after surgical revascularization. Pediatrics. 2005;116(5):e694–701.

21. Hankinson TC, Bohman L-E, Heyer G, et al. Surgical treatment of moyamoya syndrome in patients with sickle cell anemia: outcome following encephaloduroarteriosynangiosis. J Neurosurg. 2008;1:211–6.
22. Dobson SR, Holden KR, Nietert PJ, et al. Moyamoya syndrome in childhood sickle cell disease: a predictive factor for recurrent cerebrovascular events. Blood. 2002;99(9):3144–50.
23. Smith L. Understanding and treating moyamoya disease in children. Neurosurg Focus. 2009;26(4):E4.
24. Imaizumi T, Hayashi K, Saito K, Osawa M, Fukuyama Y. Long-term outcomes of pediatric moyamoya disease monitored to adulthood. Pediatr Neurol. 1998;18(4):321–5. doi:10.1016/S0887-8994(97)00209-9.
25. Kurokawa T, Tomita S, Ueda K, et al. Prognosis of occlusive disease of the circle of Willis (moyamoya disease) in children. Pediatr Neurol. 1985;1(5):274–7. doi:10.1016/0887-8994(85)90027-X.
26. Hallemeier CL, Rich KM, Grubb RL, et al. Clinical features and outcome in North American adults with moyamoya phenomenon. Stroke. 2006;37(6):1490–6. doi:10.1161/01.STR.0000221787.70503.ca.
27. Karzmark P, Zeifert PD, Tan S, Dorfman LJ, Bell-Stephens TE, Steinberg GK. Effect of moyamoya disease on neuropsychological functioning in adults. Neurosurgery. 2008;62(5):1048–51. doi:10.1227/01.neu.0000325866.29634.4c.
28. Imaizumi C, Imaizumi T, Osawa M, Fukuyama Y, Takeshita M. Serial intelligence test scores in pediatric moyamoya disease. Neuropediatrics. 1999;30(6):294–9. doi:10.1055/s-2007-973508.
29. Weinberg DG, Rahme RJ, Aoun SG, Batjer HH, Bendok BR. Moyamoya disease: functional and neurocognitive outcomes in the pediatric and adult populations. Neurosurg Focus. 2011;30(6):E21. doi:10.3171/2011.3.FOCUS1150.
30. Kawaguchi S, Okuno S, Sakaki T. Effect of direct arterial bypass on the prevention of future stroke in patients with the hemorrhagic variety of moyamoya disease. J Neurosurg. 2000;93(3):397–401. doi:10.3171/jns.2000.93.3.0397.
31. Abla AA, Gandhoke G, Clark JC, et al. Surgical outcomes for moyamoya angiopathy at barrow neurological institute with comparison of adult indirect encephaloduroarteriosynangiosis bypass, adult direct superficial temporal artery-to-middle cerebral artery bypass, and pediatric bypass: 154 revas. Neurosurgery. 2013;73(3):430–9. doi:10.1227/NEU.0000000000000017.
32. Matsushima T, Inoue T, Ikezaki K, et al. Multiple combined indirect procedure for the surgical treatment of children with moyamoya disease. A comparison with single indirect anastomosis and direct anastomosis. Neurosurg Focus. 1998;5(5):e4. doi:10.3171/foc.1998.5.5.7.
33. Matsushima T, Inoue T, Suzuki SO, Fujii K, Fukui M, Hasuo K. Surgical treatment of moyamoya disease in pediatric patients - comparison between the results of indirect and direct revascularization procedures. Neurosurgery. 1992;31(3):401–5.
34. Ueki K, Meyer FB, Mellinger JF. Moyamoya disease: the disorder and surgical treatment. Mayo Clin Proc. 1994;69(8):749–57. doi:10.1016/S0025-6196(12)61094-5.
35. Iwama T, Hashimoto N, Yonekawa Y. The relevance of hemodynamic factors to perioperative ischemic complications in childhood moyamoya disease. Neurosurgery. 1996;38(6):1120–6.
36. Nomura S, Kashiwagi S, Uetsuka S, Uchida T, Kubota H, Ito H. Perioperative management protocols for children with moyamoya disease. Childs Nerv Syst. 2001;17(4–5):270–4. doi:10.1007/s003810000407.

Chapter 14
Vascular Diseases Attributable to *RNF213* Other Than Moyamoya Disease

Satoru Miyawaki, Hideaki Imai, and Nobuhito Saito

Abstract *Ring finger protein 213* (*RNF213*) has been reported to have association with various vascular diseases other than moyamoya disease (MMD). MMD-associated genetic variant *RNF213* c.14576G>A had association with various degrees of intracranial major artery stenotic lesions including intracranial atherosclerosis (ICAS). *RNF213* c.14576G>A could be the candidate genetic variant for this prevalence of ICAS in East Asian population. The presence of *RNF213* c.14576G>A may associate with high blood pressure. Thus, *RNF213* c.14576G>A could be a robust genetic risk indicator for ischemic stroke in East Asian population. *RNF213* c.14576G>A may also have some association with quasi-MMD in East Asian population. Associations of *RNF213* with fibromuscular dysplasia and intracranial aneurysm have also been reported in European population. These results indicate that *RNF213* has a fundamental role in the pathophysiology of various vascular diseases.

Keywords Intracranial atherosclerosis • Quasi-moyamoya disease • Fibromuscular dysplasia • Intracranial aneurysm • Risk allele for ischemic stroke

14.1 Introduction

There have been several reports showing the association of *ring finger protein 213* (*RNF213*) with various vascular diseases other than moyamoya disease (MMD). In this chapter, vascular diseases attributable to *RNF213* other than MMD are reviewed.

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14.2 Intracranial Atherosclerosis

Steno-occlusive lesion of intracranial major artery (for instance, internal carotid artery, anterior cerebral artery, middle cerebral artery, and so on) is one of the main causes of ischemic stroke. Severe steno-occlusive lesion of intracranial major artery may highly lead to ischemic stroke, the annual recurrence rate of which reported to be 10–24% even under medication [1–3].

The pathogenesis of intracranial major artery stenotic lesions usually involves atherosclerosis or cardiac embolism, occasionally MMD, vasculitis, or dissection and rarely autoimmune diseases and others. The most common cause of intracranial major artery stenotic lesions is atherosclerosis, caused by acquired factors, such as hypertension, diabetes mellitus, dyslipidemia, and smoking. Intracranial atherosclerosis (ICAS) has been reported to occur more often in Asian population, including the Japanese population, than in European population, suggesting the existence of a potential genetic factor [4–6].

There is a strict diagnostic criteria for MMD [7]. The diagnostic criteria consist of imaging studies and diagnosis by exclusion. In short, the criteria consist of the following three conditions:

1. The presence of a progressive stenotic lesion at the terminal of the internal carotid artery and the development of moyamoya vessels in the basal ganglia as collateral circulation
2. The presence of the abovementioned finding on both sides
3. Lack of underlying diseases that may cause arteriostenosis (e.g., chromosomal disorder, genetic disease, inflammatory disease, injury, and tumor)

However, in clinical practice, it is sometimes difficult to distinguish MMD clearly from other pathogenesis, especially atherosclerosis. The intracranial major arteries undergo atherosclerotic changes to some degree, particularly in the elderly; therefore, when intracranial major artery stenotic lesions are discovered in the elderly, it cannot be easily distinguished whether the cause is MMD or atherosclerosis.

In addition, previous clinical studies revealed that a wide spectrum of phenotypes could occur within a family unit, with some individuals showing the typical phenotype of MMD such as bilateral stenosis/occlusion of the terminal portion of the internal carotid arteries, some showing only unilateral or middle cerebral artery stenosis/occlusion, and others with no abnormalities. These symptoms along with the family history suggest that these patients have MMD. In other words, the phenotypic spectrum of MMD appears to be wider than that allowed by the current diagnostic criteria. However, when such cases are sporadically found, they are not being categorized as MMD, and in most cases, they will be diagnosed as ICAS.

Thus, we hypothesized the MMD-associated genetic variant *RNF213* c.14576G>A (rs112735431) is involved in various phenotypes of intracranial major artery stenotic lesions that do not meet the diagnostic criteria for MMD. In other words, we predicted that *RNF213* c.14576G>A is a genetic factor for intracranial

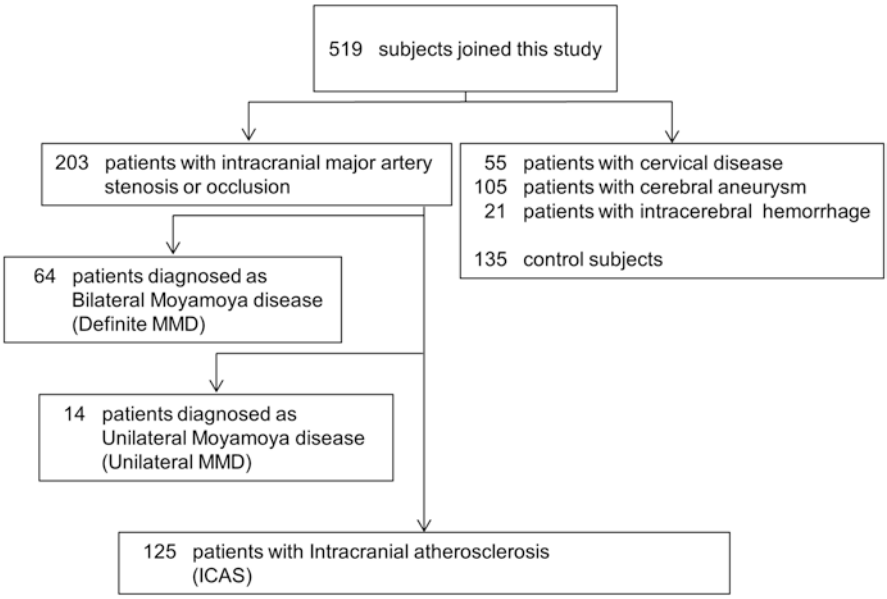

Fig. 14.1 Differential diagnosis algorithm and clinical profiles of the study participants

major artery stenosis of varying degrees, including atherosclerosis, whose cause has been previously attributed to acquired factors. To examine this hypothesis, we conducted association studies of *RNF213* c.14576G>A in various cerebrovascular diseases [8, 9].

An association study of *RNF213* c.14576G>A variant with various phenotypes of cerebrovascular diseases was conducted. Occurrence rate of *RNF213* c.14576G>A variant in patients with ICAS compared with the occurrence in patients with MMD and other cerebrovascular diseases associated with extracranial carotid atherosclerosis (ECAS), cerebral aneurysm, and intracerebral hemorrhage (ICH) as control groups.

The algorithm and profile of the differential diagnosis of these phenotypes are shown in Fig. 14.1. The result of the association study is shown in Table 14.1. There was a significant association between the *RNF213* c.14576G>A variant and the MMD group ($p < 0.0001$, odds ratio 320.4, 95% CI 68.6–1494.4). In addition, there was a significant association between the variant and the unilateral MMD group ($p < 0.0001$, odds ratio 88.6, 95% CI 15.3–511.3) and the ICAS group ($p < 0.0001$, odds ratio 20.0, 95% CI 4.68–86.2). Meanwhile, there was no significant association between the variant and the other groups (ECAS, cerebral aneurysm, and ICH groups).

From these analysis, the *RNF213* c.14576G>A variant was revealed to be associated with stenotic lesions of the intracranial major arteries in various degrees. We discovered a significant association between the MMD-related genetic variant

Table 14.1 Comparison of the occurrence rate of c.14576G>A variant in each phenotype group with the control group

	Definite MMD	Unilateral MMD	ICAS	ECAS	Cerebral aneurysm	ICH	Control
Rate of occurrence, n^a (%)	53/64 (82.8)	8/14 (57.1)	29/125 (23.2)	1/55 (1.8)	1/105 (0.9)	0/21 (0)	2/135 (1.5)
p value	<0.0001	<0.0001	<0.0001	1.00	1.00	1.00	
OR	320.4	88.6	20.0	1.23	0.63	0	
95% CI	68.6–1494.4	15.3–511.3	4.68–86.2	0.10–13.8	0.05–7.1		

MMD indicates moyamoya disease, *ICAS* intracranial atherosclerosis, *ECAS* extracranial carotid atherosclerosis, *ICH* intracranial hemorrhage, *OR* odds ratio, *CI* confidence interval
[a]No. of patients with c.14576G>A variant (G/A + A/A)/total no. of patients

RNF213 c.14576G>A and stenotic lesions in the intracranial major arteries in various phenotypes, including unilateral MMD and ICAS, in addition to MMD [8, 9].

This result was also confirmed by other groups. Bang et al. reported the significant association of *RNF213* c.14576G>A variant and ICAS in Korean population [10, 11].

As mentioned before, several previous reports have shown that Asians experience development of ICAS with significantly higher rates than whites, suggesting that a genetic factor peculiar to Asians might be responsible. The c.14576G>A variant in *RNF213* could be the candidate genetic variant for this epidemiological difference because the *RNF213* c.14576G>A variant is present in ≈2% of East Asian populations, a relatively higher rate compared with European population [12, 13]. The presence of *RNF213* c.14576G>A may associate with high blood pressure. Thus, *RNF213* c.14576G>A could be a robust genetic risk allele for ischemic stroke in East Asian population.

14.3 Quasi-Moyamoya Disase

Quasi-moyamoya disease (quasi-MMD) or moyamoya syndrome defined as a vasculopathy which is angiographically equivalent to MMD and has underlying comorbidities such as neurofibromatosis type 1 (NF1), Down syndrome, thyroid disease, cranial irradiation, or sickle cell anemia. Association study of *RNF213* c.14576G>A variant and quasi-MMD was reported from several groups, to determine whether quasi-MMD and MMD have any common etiology or genetic background.

We reported the result of the genetic analysis of *RNF213* c.14576G>A variant in nine Japanese quasi-MMD patients. The nine patients with quasi-MMD disease included three patients with previous irradiation, two with hyperthyroidism, one with Turner syndrome, one with meningitis, one with Behçet disease, and one with

idiopathic pachymeningitis. All of the quasi-MMD patients did not have the *RNF213* c.14576G>A variant [14].

Phi et al. reported an association study of *RNF213* variant had significant association with quasi-MMD patients with NF-1. Three out of 16 quasi-MMD patients with NF-1 had the *RNF213* variant. *RNF213* variant had significant with quasi-MMD patients with NF-1 [15].

Morimoto et al. analyzed *RNF213* c.14576G>A variant in 18 quasi-MMD patients. Underlying diseases in the 18 quasi-MMD patients are shown in Table 14.2. The variant was found in 12 of 18 quasi-MMD patients. The frequency of *RNF213* c.14576G>A carriers was significantly higher in quasi-MMD cases than in controls [16].

The result of the above three studies are summarized in Table 14.2. From these results, *RNF213* variant is present in quasi-MMD to some extent, although lower rate than that in the definite MMD. A larger study is needed to analyze the effect of the *RNF213* c.14576G>A genotype on the pathogenesis and the clinical characteristics of quasi-MMD.

14.4 Other Vascular Diseases

Association of *RNF213* variant and other vascular diseases has also been reported.

Kiando et al. reported the association with *RNF213* and fibromuscular dysplasia in European population, which is characterized by the vasculopathy leading to stenosis, aneurysm, and dissection, mainly of renal arteries and carotids [17].

Zhou et al. reported the association of *RNF213* and intracranial aneurysms in the French-Canadian population [18]. They found several deleterious variants in the AAA domains, whereas other studies focused on MMD affected individuals did not [19, 20]. They assumed that variants in different locations of *RNF213* act as risk factors for different cerebrovascular disorders by affecting gene function differently. Whereas Cecchi et al. [19] suggested that the C-terminal domain of *RNF213* is the main risk region for MMD, exon 29, which encodes AAA domains, might be the risk region for intracranial aneurysms. Thus, *RNF213* variations might be associated with different vascular disorders in different populations.

14.5 Conclusion

Association of *RNF213* and various vascular diseases implies the important and fundamental role of *RNF213* in the pathophysiology of various vascular diseases. Molecular functional analysis of *RNF213* may lead to new treatment for stroke. For East Asian population, *RNF213* c.14576G>A may be a robust genetic risk allele for

Table 14.2 Occurrence rate of c.14576G>A variant in quasi-moyamoya disease in the previous reports

	Miyawaki et al. [14]		Phi et al.		Morimoto et al.	
	Baseline diseases	Rate of occurrence n^a (%)	Baseline diseases	Rate of occurrence n^a (%)	Baseline diseases	Rate of occurrence n^a (%)
	Irradiation	0/3	Neurofibromatosis 1	3/16	Neurofibromatosis 1	1/4
	Hyperthyroidism	0/2			Hyperthyroidism	2/4
	Turner syndrome	0/1			Down syndrome	1/2
	Meningitis	0/1			Kawasaki disease	2/2
	Behçet disease	0/1			Rheumatoid arthritis	2/2
	Idiopathic pachymeningitis	0/1			Systemic lupus erythematosus	1/1
					Noonan syndrome	1/1
					Autoimmune pancreatitis	1/1
					Sjögren's syndrome and Hashimoto's thyroiditis	1/1
Total		0/9		3/16 (18.7)		12/18 (66.6)

[a]No. of patients with c.14576G>A variant (G/A + A/A)/total no. of patients

ischemic stroke. Preventive treatment for stroke, such as blood pressure management, and image screening, such as MR angiography, for intracranial cerebral arterial lesion would be very important, especially for individuals with this specific allele in this population.

Reference

1. Prognosis of patients with symptomatic vertebral or basilar artery stenosis. The Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) Study Group. Stroke. 1998; 29:1389–92.
2. Thijs VN, Albers GW. Symptomatic intracranial atherosclerosis: outcome of patients who fail antithrombotic therapy. Neurology. 2000;55:490–7.
3. Kasner SE, Chimowitz MI, Lynn MJ, Howlett-Smith H, Stern BJ, Hertzberg VS, et al. Predictors of ischemic stroke in the territory of a symptomatic intracranial arterial stenosis. Circulation. 2006;113:555–63. doi:10.1161/CIRCULATIONAHA.105.578229.
4. Caplan LR, Gorelick PB, Hier DB. Race, sex and occlusive cerebrovascular disease: a review. Stroke. 1986;17:648–55.
5. Arenillas JF. Intracranial atherosclerosis: current concepts. Stroke. 2011;42:S20–3. doi:10.1161/STROKEAHA.110.597278.
6. Suri MF, Johnston SC. Epidemiology of intracranial stenosis. J Neuroimaging. 2009;19(Suppl 1):11S–6S. doi:10.1111/j.1552-6569.2009.00415.x.
7. Guidelines for diagnosis and treatment of moyamoya disease (Spontaneous Occlusion of the Circle of Willis). Neurol Med Chir (Tokyo). 2012;52:245–66.
8. Miyawaki S, Imai H, Takayanagi S, Mukasa A, Nakatomi H, Saito N. Identification of a genetic variant common to moyamoya disease and intracranial major artery stenosis/occlusion. Stroke. 2012;43:3371–4. doi:10.1161/strokeaha.112.663864.
9. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, et al. Genetic variant *RNF213* c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke. 2013;44:2894–7. doi:10.1161/STROKEAHA.113.002477.
10. Bang OY, Ryoo S, Kim SJ, Yoon CH, Cha J, Yeon JY, et al. Adult Moyamoya disease: a burden of intracranial stenosis in East Asians? PLoS One. 2015;10:e0130663. doi:10.1371/journal.pone.0130663.
11. Bang OY, Chung JW, Cha J, Lee MJ, Yeon JY, Ki CS, et al. A polymorphism in *RNF213* is a susceptibility gene for intracranial atherosclerosis. PLoS One. 2016;11:e0156607. doi:10.1371/journal.pone.0156607.
12. Liu W, Hitomi T, Kobayashi H, Harada KH, Koizumi A. Distribution of moyamoya disease susceptibility polymorphism p.R4810K in *RNF213* in East and Southeast Asian populations. Neurol Med Chir (Tokyo). 2012;52:299–303.
13. Cao Y, Kobayashi H, Morimoto T, Kabata R, Harada KH, Koizumi A. Frequency of *RNF213* p.R4810K, a susceptibility variant for moyamoya disease, and health characteristics of carriers in the Japanese population. Environ Health Prev Med. 2016:387–90. doi:10.1007/s12199-016-0549-8.
14. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Nakatomi H, et al. Genetic analysis of *RNF213* c.14576G>A variant in nonatherosclerotic quasi-moyamoya disease. J Stroke Cerebrovasc Dis. 2015;24:1075–9. doi:10.1016/j.jstrokecerebrovasdis.2015.01.005.
15. Phi JH, Choi JW, Seong MW, Kim T, Moon YJ, Lee J, et al. Association between moyamoya syndrome and the *RNF213* c.14576G>A variant in patients with neurofibromatosis Type 1. J Neurosurg Pediatr. 2016;17:717–22. doi:10.3171/2015.10.PEDS15537.

16. Morimoto T, Mineharu Y, Kobayashi H, Harada KH, Funaki T, Takagi Y, et al. Significant association of the *RNF213* p.R4810K polymorphism with quasi-moyamoya disease. J Stroke Cerebrovasc Dis. 2016;25:2632–6. doi:10.1016/j.jstrokecerebrovasdis.2016.07.004.
17. Kiando SR, Barlassina C, Cusi D, Galan P, Lathrop M, Plouin PF et al. Exome sequencing in seven families and gene-based association studies indicate genetic heterogeneity and suggest possible candidates for fibromuscular dysplasia. J Hypertens 2015;33:1802–10. doi: 10.1097/hjh.0000000000000625; discussion 10.
18. Zhou S, Ambalavanan A, Rochefort D, Xie P, Bourassa CV, Hince P, et al. *RNF213* Is Associated with Intracranial Aneurysms in the French-Canadian Population. Am J Hum Genet. 2016;99:1072–85. doi:10.1016/j.ajhg.2016.09.001.
19. Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, et al. *RNF213* rare variants in an ethnically diverse population with Moyamoya disease. Stroke. 2014;45:3200–7. doi:10.1161/STROKEAHA.114.006244.
20. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of moyamoya disease and associated health risks explored through *RNF213*. Environ Health Prev Med. 2016;21:55–70. doi:10.1007/s12199-015-0498-7.

Part V
Epilogue

Chapter 15
Future Clinical Perspectives on *RNF213* in Moyamoya Disease

Shigeo Kure

Abstract Early diagnosis and early surgical intervention are critical for improving the prognosis of moyamoya disease (MMD). For early diagnosis, it is necessary to develop biomarkers that can be used for the evaluation of MMD risk. In this chapter, biomarkers such as clinical symptoms, radiographic findings, protein markers, and genetic markers are discussed. *RNF213* is the first susceptibility gene for MMD identified in East Asian countries. Genetic testing for the c.14576G>A variant in *RNF213* has been shown to be useful for the evaluation of risk and the prediction of the severity of MMD. We are currently genotyping the c.14576G>A variant and providing genetic counseling to improve patients' understanding of the disease risk. How the *RNF213* variant causes the vascular abnormalities of MMD remains unknown. The elucidation of the physiological function of the *RNF213* gene product may lead to the development of a new drug that is effective for the treatment or prevention of MMD.

Keywords Biomarkers of MMD • Genetic testing of *RNF213* • Gene-dose effect of *RNF213* • Evaluation of risk of MMD by genetic testing • Genetic counseling with *RNF213* genetic testing

15.1 Present Clinical Problems

Moyamoya disease (MMD) is a rare cerebrovascular disease in children and adults, which is characterized by steno-occlusion in the terminal portion of the internal carotid artery (ICA) and an abnormal fine vascular network at the base of the brain [1]. The only established therapy for MMD is surgical intervention involving direct and/or indirect revascularization [2]. The most important goal of surgical

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revascularization is to prevent cerebral infarction by improving blood flow. Because MMD is more progressive in children than in adults, surgical revascularization is indicated in most pediatric patients with MMD. To improve the prognosis, it is therefore very important to establish an early diagnosis and active intervention before severe brain damage occurs. The diagnosis of MMD in suspected patients is commonly confirmed by MRI and MR angiography (MRA). For early diagnosis, the following two issues are important: (1) how to identify individuals with a high risk of MMD and (2) how to facilitate MRI/MRA testing among high-risk individuals. In this chapter, we will discuss clinical symptoms and biomarkers that facilitate the early diagnosis of MMD.

15.2 Clinical Symptoms Leading to Early Diagnosis

Pediatric patients usually develop transient ischemic attack (TIA) as initial symptom, thus providing an opportunity for early diagnosis. However, TIA symptoms, such as short syncope after crying in infants or younger children, tend to be overlooked. Much more attention should be paid to TIA in the pediatric period, because it sometimes takes several years for some patients with TIA to receive a diagnosis of MMD. Crescendo TIA, which represents two or more TIAs in a week, is a high-risk symptom of MMD. In younger children, sedation is usually needed to perform MR angiography, which may delay the timing of MR angiography. In a brain dock, MMD is sometimes diagnosed in asymptomatic adults by MRI/MRA testing. Follow-up MRI/MRA testing would provide valuable information regarding the timing and methods of surgical intervention.

15.3 Radiographic Makers

The MRI/MRA test provides information on the abnormality of cerebral arteries, the parenchyma of the brain, and cerebral perfusion, which are essential for follow-up. The important findings in MRI/MRA testing used for the diagnosis of MMD are (1) stenosis or occlusion of the terminal internal cervical artery (ICA) and/or the proximal part of the anterior cervical artery (ACA) and/or middle cervical artery (MCA) and (2) visualization of an abnormal arterial network in the vicinity of the steno-occlusive lesions. If these findings are observed bilaterally, the diagnosis is “definite MMD.” If they are unilateral, the diagnosis is “probable MMD.” Asymptomatic microbleeds are usually observed in MRI imaging in adult patients but rarely in pediatric patients. The microbleeds may be a sign of a high risk of hemorrhagic stroke [3]. In FLAIR imaging, prominent leptomeningeal collaterals result in vivid contrast enhancement and high signals due to slow flow, which are called the “ivy sign” [4].

15.4 Protein Markers

It would be beneficial to develop blood protein markers that can be detected in the early phase of MMD. Many proteins in the blood have been reported to have significantly different levels in MMD patients compared with controls: alpha1 antitrypsin [5], MMP-3, MMP-9, TIMP-1, TIMP-2, VEGF, PDGF-BB, MCP-1, IL-1 [6], ANCA [7] and SDF-1 alpha [8]. However, none have been studied in blood samples obtained from patients prior to the onset of MMD. It therefore remains to be clarified which proteins can be used as predictive markers. A recent mass spectrometric analysis has identified a novel 4.4-kDa protein in the cerebrospinal fluid that correlates with collateral development after revascularization surgery [9]; this protein may be a useful marker in the follow-up period.

15.5 Genetic Markers

15.5.1 Genetic Factors of MMD

The involvement of genetic factors in the etiology of MMD has been strongly suggested by the high concordance rate observed in monozygotic twins, the 10–15 % portion of the MMD family with multiple affected members, and strong ethnicity-related effects with a high prevalence in East Asian countries and a low prevalence in Western countries [10]. Several genome-wide linkage analyses performed in Japan have identified five MMD loci: 3p24.2–p26 [11], 6p25 [12], 8p23 [13], 12p12 [13], and 17q25 [14, 15].

15.5.2 Identification of RNF213 as a Susceptibility Gene for MMD

In 2011, we performed a genome-wide association study (GWAS) using Japanese MMD patients and control individuals and found a very strong association on chromosome 17q25 (Fig. 15.1). This strong association was present in the SNPs located in the *RNF213* gene region [16]. Sequencing analysis of the *RNF213* gene in MMD patients identified a risk variant, p.R4859K (c.14576G>A on the basis of the National Center for Biotechnology Information NCBI Reference sequence number NM_020914.4). The p.R4859K variant has a surprisingly high prevalence of 95 % in familial cases and 73 % in nonfamilial cases. The prevalence of the SNP is 1.4 % in the normal population, thus suggesting that the SNP carriers are at high risk for MMD (odds ratio=190.8, 95 % confidence interval=71.7–507.9). In the same year, a research group from Kyoto University independently identified *RNF213* as a MMD gene by linkage analysis and by sequencing analysis of the risk SNP

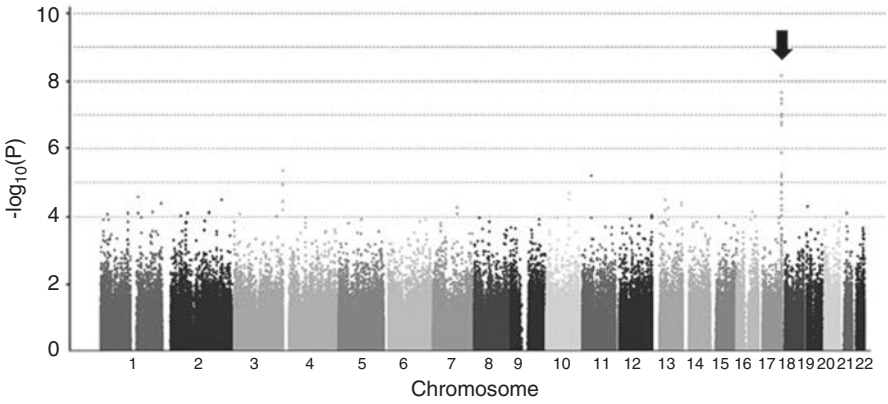

Fig. 15.1 The result of GWAS with the MMD patients and control individuals is shown in Manhattan plot. A strong association is observed on chromosome 7p25 (*arrow*) (This figure is cited from Ref. [16])

p.R4810K (c.14429G>A on the basis of NCBI reference sequence number NM_001256071) [17]. *RNF213* exhibits tissue-specific alternative splicing with or without exon 4, which encodes 49 amino acids [17], thus resulting in different amino acid numbers (pR4859K and p.R4810K) and different cDNA nucleotide numbers (c.14576G>A and c.14429G>A) for the same SNP. This SNP is prevalent in East Asia but is extremely rare in Western countries, thus suggesting that it can be used for the estimation of genetic risk for MMD in East Asian countries.

15.5.3 Gene-Dose Effect of the RNF213 Mutation

Miyatake et al. have studied the correlation between the *RNF213* genotype of c.14576G>A (c.14429G>A) variant and clinical features [18]. As shown in Fig. 15.2, in patients homozygous for c.14576G>A, 60 % were diagnosed with MMD before the age of 4, and all of them had infarctions as the first symptom. Infarctions upon initial presentation and the involvement of posterior cerebral arteries, both known as poor prognostic factors for MMD, were significantly more prevalent among homozygotes than in heterozygotes and the wild type, thus suggesting that the *RNF213* mutant allele has a gene-dose effect. This effect has been illustrated by a sibling case of a homozygous brother and a heterozygous sister for the c.14576G>A variant [19]. The brother presented with initial symptoms at 2 years of age, and his neurological deficits were severe. In contrast, the sister showed initial symptoms of TIA at 17 years of age and a mild clinical course with a limited distribution of vasculopathy. The genotyping of the c.14576G>A variant would be useful not only for the evaluation of risk for MMD but also for the prediction of the severe type of MMD.

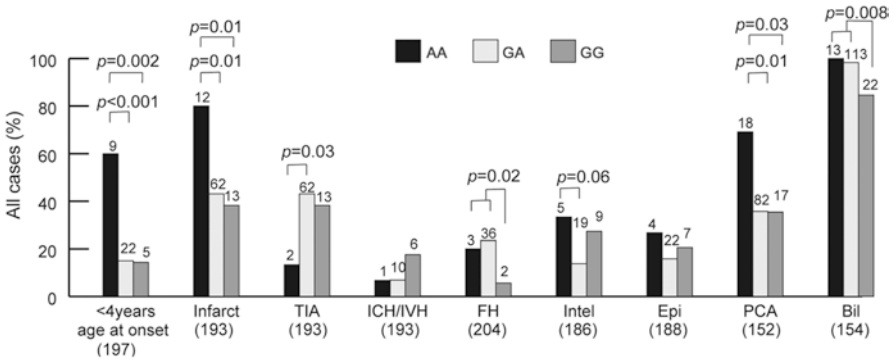

Fig. 15.2 The clinical characteristics of MMD for the three groups of patients (a total of 204 patients) with either the homozygote (AA), heterozygote (GA), or wild type (GG) of the c.14576G>A variant. The numbers of total patients with clinical records regarding either the presence or absence of each characteristic are indicated *below* the *bars*, and the numbers of patients in each group are indicated *above* the respective *bars*. *Bil* bilateral vasculopathy, *Epi* epilepsy, *FH* with family history, *ICH/IVH* intracranial hemorrhage/intraventricular hemorrhage, *Infarct* infarction, *Intel* intellectual impairment, *PCA* posterior cerebral artery involvement (This figure is cited from Ref. [18])

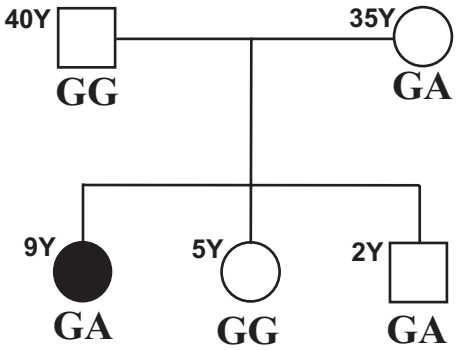

Fig. 15.3 A typical case of MMD family who visited Tohoku University Hospital for the genetic testing and genetic counseling. The family have three children; the elder sister was diagnosed as having MMD and treated by the bypass operation at the age of 5. Age of each family member is indicated in the *left* side. The genotype of c.14576G>A variant is shown as *GA* heterozygotes, *GG* wild type (no risk allele)

15.5.4 RNF213 Genetic Testing and Genetic Counseling

Because it has been clinically useful to genotype the c.14576G>A, we began to perform genetic testing. *RNF213* is a susceptibility gene, not a causative gene, for MMD, thus making it difficult to understand the results of the genetic testing. To improve patient understanding, we have provided genetic counseling as well as genetic testing for MMD. Figure 15.3 represents a typical case of genetic

counseling. The patient's parents were anxious that MMD might develop because they had researched the heritability of MMD on the Internet. Before the genetic testing, we emphasized to the parents that a carrier of the c.14576G>A variant has a higher risk of MMD but does not always develop MMD. In the first step of the test, the affected brother was genotyped, and the results confirmed that he was heterozygous for the c.14576G>A variant. In the second step, both parents and two younger siblings were tested. The mother, but not the father, carried the risk allele, thus suggesting that the risk allele is maternal. The younger brother, but not the younger sister, was a carrier of the risk allele. We explained to the parents that a follow-up examination was no longer required for the younger sister. We also explained that the younger brother had an increased risk of MMD and recommended a yearly follow-up with MRI/MRA testing. The mother also carried the risk allele, and we recommended MRI/MRA testing with a periodic brain dock. A follow-up protocol for asymptomatic carriers of the risk allele has not been established. The interval and duration of MRI/MRA testing should be discussed for the follow-up of pediatric as well as adult patients.

15.6 Conclusion

The c.14576G>A variant in *RNF213* is a useful biomarker in genetic testing to evaluate the risk for MMD in East Asia; it may facilitate the early diagnosis of MMD. Clarification of the physiological function of the *RNF213* gene product would be useful because it might lead to the development of a new drug that is effective for the treatment or prevention of MMD. The development of new biomarkers would facilitate a more accurate evaluation of the risk and predicted onset of MMD.

References

1. Suzuki J, Takaku A. Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain. Arch Neurol. 1969;20(3):288–99.
2. Fujimura M, Tominaga T. Current status of revascularization surgery for Moyamoya disease: special consideration for its 'internal carotid-external carotid (IC-EC) conversion' as the physiological reorganization system. Tohoku J Exp Med. 2015;236(1):45–53.
3. Kuroda S, Kashiwazaki D, Ishikawa T, Nakayama N, Houkin K. Incidence, locations, and longitudinal course of silent microbleeds in moyamoya disease: a prospective T2*-weighted MRI study. Stroke. 2013;44(2):516–8.
4. Chabbert V, Ranjeva JP, Sevely A, Boetto S, Berry I, Manelfe C. Diffusion- and magnetisation transfer-weighted MRI in childhood moya-moya. Neuroradiology. 1998;40(4):267–71.
5. Amano T, Inoha S, Wu CM, Matsushima T, Ikezaki K. Serum alpha1-antitrypsin level and phenotype associated with familial moyamoya disease. Childs Nerv Syst. 2003;19(9):655–8.
6. Kang HS, Kim JH, Phi JH, Kim YY, Kim JE, Wang KC, et al. Plasma matrix metalloproteinases, cytokines and angiogenic factors in moyamoya disease. J Neurol Neurosurg Psychiatry. 2010;81(6):673–8.

7. Yanagawa Y, Sugiura T, Suzuki K, Okada Y. Moyamoya disease associated with positive findings for rheumatoid factor and myeloperoxidase-anti-neutrophil cytoplasmic antibody. West Indian Med J. 2007;56(3):282–4.
8. Ni G, Liu W, Huang X, Zhu S, Yue X, Chen Z, et al. Increased levels of circulating SDF-1alpha and CD34+ CXCR4+ cells in patients with moyamoya disease. Eur J Neurol. 2011;18(11):1304–9.
9. Maruwaka M, Yoshikawa K, Okamoto S, Araki Y, Sumitomo M, Kawamura A, et al. Biomarker research for moyamoya disease in cerebrospinal fluid using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. J Stroke Cerebrovasc Dis. 2015;24(1):104–11.
10. Kure S. Genetics-overview. In: Cho BK, Tominaga T, editors. Moyamoya disease update. Tokyo: Springer; 2010. p. 41–5.
11. Ikeda H, Sasaki T, Yoshimoto T, Fukui M, Arinami T. Mapping of a familial moyamoya disease gene to chromosome 3p24.2-p26. Am J Hum Genet. 1999;64(2):533–7.
12. Inoue TK, Ikezaki K, Sasazuki T, Matsushima T, Fukui M. Linkage analysis of moyamoya disease on chromosome 6. J Child Neurol. 2000;15(3):179–82.
13. Sakurai K, Horiuchi Y, Ikeda H, Ikezaki K, Yoshimoto T, Fukui M, et al. A novel susceptibility locus for moyamoya disease on chromosome 8q23. J Hum Genet. 2004;49(5):278–81.
14. Yamauchi T, Tada M, Houkin K, Tanaka T, Nakamura Y, Kuroda S, et al. Linkage of familial moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. Stroke. 2000;31(4):930–5.
15. Mineharu Y, Liu W, Inoue K, Matsuura N, Inoue S, Takenaka K, et al. Autosomal dominant moyamoya disease maps to chromosome 17q25.3. Neurology. 2008;70(24 Pt 2):2357–63.
16. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, et al. A genome-wide association study identifies *RNF213* as the first Moyamoya disease gene. J Hum Genet. 2011;56(1):34–40.
17. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, et al. Identification of *RNF213* as a susceptibility gene for moyamoya disease and its possible role in vascular development. PLoS One. 2011;6(7):e22542.
18. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576G>A variant of *RNF213* predicts early-onset and severe form of moyamoya disease. Neurology. 2012;78(11):803–10.
19. Miyatake S, Touho H, Miyake N, Ohba C, Doi H, Saitsu H, et al. Sibling cases of moyamoya disease having homozygous and heterozygous c.14576G>A variant in *RNF213* showed varying clinical course and severity. J Hum Genet. 2012;57(12):804–6.