Impact of RNF213 Founder Polymorphism (p.R4810K) on the Postoperative Development of Indirect Pial Synangiosis After Direct/indirect Combined Revascularization Surgery for Adult Moyamoya Disease.

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Research Article

Keywords: Moyamoya, Magnetic Resonance Imaging, Polymorphism, Revascularization, RNF213, Vascular disorders

Posted Date: August 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-781282/v1

Version of Record: A version of this preprint was published at Neurosurgical Review on February 7th, 2022. See the published version at https://doi.org/10.1007/s10143-022-01749-9.

Abstract

Background

Direct superficial temporal artery (STA) to middle cerebral artery (MCA) anastomosis combined with indirect pial synangiosis provides favorable surgical collaterals for Moyamoya disease (MMD), especially in adults; however, factors leading to the development of each direct and indirect collateral are not well documented.

Objective

To investigate the association between *RNF213* founder polymorphism (p.R4810K) and each direct and indirect collateral development.

Methods

By qualitative and quantitative evaluations of direct and indirect surgical collaterals using time-of-flight MR angiography, postoperative development of each type of bypass was evaluated independently into two categories. Multivariate logistic regression analysis was performed to study the contributing factors for the development of each surgical collateral.

Results

Excellent development of postoperative direct and indirect bypass was observed in 65 hemispheres (70%) by qualitative evaluation, which was confirmed by quantitative evaluation. Multivariate logistic regression analysis of excellent indirect bypass development revealed a significant positive correlation with the p.R4810K (odds ratio, OR4.0; 95%-confidence interval, CI 1.2-16), advanced MR angiographic stage (OR9.5; 95%CI 1.7-73), and preoperative middle meningeal artery caliber (OR6.8; 95%CI 1.8-35), but a significant negative correlation was found with the excellent direct bypass development (OR0.17; 95%CI 0.03-0.75). No significant correlation was observed between excellent direct bypass development and the p.R4810K (OR0.95; 95%CI 0.37-2.4).

Conclusion

Excellent development of indirect collaterals after STA-MCA anastomosis combined with indirect pial synangiosis occurs more frequently in adult MMD with the *RNF213* founder polymorphism, suggesting a role of the p.R4810K variant for marked in-growth of indirect collaterals and the utility of preoperative genetic analysis.

Introduction

Moyamoya disease (MMD) is characterized by progressive stenosis of the terminal portion of the internal carotid arteries, accompanied by the formation of abnormally dilated, fragile perforators at the base of the brain. [6, 17, 28, 30] Direct superficial temporal artery (STA) to middle cerebral artery (MCA) anastomosis is accepted worldwide as the primary treatment of choice aiming at improving cerebral blood flow and surgical revascularization for symptomatic ischemic and hemorrhagic presentations of MMD. [16, 24] While direct STA-MCA anastomosis offers the advantage of immediate revascularization, indirect bypass effectively induces the in-growth of new collaterals to the underlying cerebral cortex overtime. [17, 28] In the combined setting using both direct and indirect bypass, the advantages of both bypass procedures are expected, with a perioperative

stroke risk of 4.7% per surgery but a favorable long term clinical outcome. [14, 19] Several studies demonstrated a reciprocal relationship between the direct and indirect bypass with a wider extent of surgical revascularization in the context of a combined setting. [1, 2, 33, 34] Very recently, the association between a *RNF213* founder mutation for east Asian MMD (p.R4810K) [13, 21] and postoperative development of surgical collaterals has been demonstrated,[27] however, it is still obscure for which type of surgical collateral (i.e., direct or indirect or both?) the *RNF213* founder mutation is responsible.

The extent of revascularization after Moyamoya bypass surgery is traditionally examined by catheter carotid angiography. [2, 22] In the early 2000s, Yoon et al.[35] and Honda et al.[22] reported the usefulness of magnetic resonance (MR) angiography to evaluate the development of the external carotid artery tributaries, including the STA, middle meningeal artery (MMA), and deep temporal artery (DTA) after direct and/or indirect bypass surgery for MMD. Recent studies using time-of-flight (TOF) MR angiography or its source image provided insights into the discernable and sequential roles in direct and/or indirect bypass in MMD. [1, 34] To comprehensively investigate the clinical and genetic factors associated with the induction of each direct and indirect surgical collaterals, we retrospectively examined our institutional records and MR angiography for adult patients undergoing combined direct/indirect bypass for MMD.

Materials And Methods

Below are the main methods necessary to comprehend the results. Details were provided in the online-only supplementary information.

Study Population

This study included all adult MMD patients (>16 years of age at the surgery) who consented to genetic analysis and underwent repeat MR imaging within 3 years after combined direct/indirect bypass [9, 18] between 2005 and 2019 at our hospital. quasi-MMD was excluded. The Houkin MR angiographical stage/grading system was used to stratify the angiographical stage of MMD (ranging 1 to 4 [most advanced]). [10] Cerebrovascular reactivity to acetazolamide was evaluated quantitatively as previously reported. [33] In accordance with an institutional review board-approved protocol (number14-053), medical records were retrospectively reviewed to gather demographic information, age at the surgery, symptoms at presentation, comorbid conditions before surgery, and results of radiographic studies (Please see supplementary table 1).

Evaluation of the Postoperative Development of Direct and Indirect Surgical Collaterals by MR angiography

Evaluation of the postoperative development of direct and indirect surgical collaterals was performed qualitatively and quantitatively using TOF MR angiography and its source images, respectively, by a previously reported protocol with minor modifications [33, 34]. In brief, MR angiography at two time points acquired before and 6 to 36 months after surgery were reviewed and compared by neurosurgeons (M. I. and T.S.) blinded to the results of genetic testing. For qualitative evaluation, [33] postoperative development of each direct and indirect surgical collaterals were dichotomized into excellent or not, respectively. Thus, the development of direct surgical collaterals was evaluated by the development of the STA, while that of indirect surgical collaterals was evaluated by the development of the MMA and DTA. For quantitative evaluation, we reviewed MR angiography source images and measured the calibers of the STA, MMA, and DTA as previously reported.[34] The caliber change ratios (CCRs) of post to preoperative calibers were calculated for each artery.

Genetic Analysis of the *RNF213* founder polymorphism (p.R4810K)

Written-informed consent was received for genetic analysis from MMD patients or their guardians. In accordance with the institutional review board-approved protocol, genetic analysis was conducted at the Department of Neurosurgery of Hokkaido University by K.T. and R.T. who were blinded to clinical data. Taqman single nucleotide polymorphism genotyping assay was employed to determine the allele type for *RNF21*3 founder mutation (p.R4810K).

Data Analysis

Continuous or rank variables were described as the mean or median with standard deviation or range. Dichotomous or categorical variables were expressed as the ratio or frequency. Continuous or rank variables were compared between two groups by the unpaired-t test or Mann-Whitney test. Dichotomous or categorical variables were compared by Fisher's exact test. For multiple comparisons, two-way repeated measures or ordinary analysis of variance followed by a post-hoc test was used, as appropriate. To assess the correlation between the excellent development of each surgical collateral and multiple clinical and genetic variables, multivariate logistic regression analysis was performed using the stepwise forward parameter selection that achieved significance levels of $P < 0.1$ in the univariate analysis. All clinical and genetic factors for the multivariate analysis are listed in the main Tables. The level of significance was set at $P < 0.05$. GraphPad Prism (version 9.1.1, San Diego, CA, USA) was used for these analyses.

Results

Study Population

During the study period, 110 adult patients with 160 hemispheres underwent STA-MCA anastomosis combined with indirect pial synangiosis using vascularized tissue, including dura mater and temporal muscle. Of these, 48 were excluded from analysis due to lack of genetic testing, missing follow-up MR imaging within 6 to 36 months after surgery, or the diagnosis of quasi-MMD (n=43, 2, and 3 patients, respectively). Consequently, 62 patients (56%) with 93 operated hemispheres (58%) were included in the analysis: 47 female and 15 male patients with an average age of 42 years. *RNF213* founder mutation (p.R4810K) was detected in 40 patients (65%) with 59 hemispheres (63%) in this series (Figure 1A). There was no significant difference in baseline characteristics between *RNF213*-mutant (MT) and -wild type (WT) groups, except in familial occurrence and a comorbid condition of hypertension (Supplementary Table. 2). A representative patient who was a heterozygote for the *RNF213* founder mutation is shown in Figure 1B-E.

Qualitative and Quantitative MR Angiography Evaluations

Of all 93 hemispheres enrolled in this study, excellent development of postoperative direct and indirect surgical collaterals was observed in 65 (70%) after the combined bypass with a mean follow-up period of 319±140 days by qualitative evaluation. In terms of the relationship of the postoperative development between direct and indirect bypass, dual/equal development was most frequently observed (62% of the operated hemisphere). Thus, postoperative direct- (STA) or indirect- (MMA and/or DTA) dominant development was observed in 44 and 14 hemispheres, respectively, when evaluated in the above-mentioned follow-up period. By quantitative MR angiography evaluation of all 93 hemispheres, a significant increase was observed in the caliber of the STA after

surgery (pre: 1.8±0.35 mm; post: 2.5±0.65 mm, P<0.0001). The caliber of the MMA and DTA also significantly increased (pre: 1.6±0.44 mm; post: 1.8±0.50 mm, P<0.0001 in MMA; pre: 0.98±0.33 mm; post: 1.6±0.60 mm, P<0.0001, respectively). The CCR of post to preoperative-calibers for the STA was significantly higher (1.5±0.42 vs 1.0±0.28, P<0.0001) in the excellent direct bypass development group than in the non-excellent group. The CCRs for the DTA (2.0±0.89 vs 1.2±0.53, P<0.0001) and MMA (1.3±0.31 vs 1.0±0.29, P=0.042) were also significantly higher in the excellent indirect bypass development group.

We further analyzed the association between direct or indirect bypass development and *RNF213* founder polymorphism (p.R4810K). Of note, excellent indirect bypass development was observed more frequently in the *RNF213*-MT group (78%, 46/59 hemispheres) than in the *RNF213*-WT group (56%, 19/34 hemispheres), with a significant difference (P = 0.035) (Figure 2A). Excellent direct bypass development was observed in 40/59 hemispheres (68%) and 24/34 hemispheres (71%) of the *RNF213*-MT and -WT groups, respectively, with no significant difference (P = 0.82). Multiple comparisons of the CCRs demonstrated a significant difference in the DTA, but not in the STA or MMA, between the two groups (Figure 2B). Thus, the CCR for the DTA was significantly higher in the *RNF213*-MT group (1.9±1.0) than in the *RNF213*-WT group (1.4±0.52, P=0.0007).

Factors Correlated with Excellent Development of Indirect and Direct Collaterals After Combined Bypass

To identify clinical and genetic factors that may underlie excellent revascularization after combined bypass in adult MMD, we examined which factors correlated with the excellent development of indirect and direct collaterals (Table 1 and 2). Multivariate logistic regression analysis for excellent indirect bypass development revealed a significant positive correlation with *RNF213* founder mutation (adjusted odds ratio (OR), 4.0), advanced MR angiographic stage (adjusted OR, 13 in stage 3; 9.5 in stage 4), and preoperative caliber of the MMA (adjusted OR, 6.8), whereas a significant negative correlation was noted with excellent direct bypass development (adjusted OR, 0.17). On the other hand, no significant correlation was observed between excellent direct bypass development and *RNF213* founder mutation. Multivariate logistic regression analysis for excellent direct bypass development revealed a significant negative correlation with excellent indirect bypass development (adjusted OR, 0.23) and the comorbid condition of dyslipidemia (adjusted OR, 0.27). Please see supplementary figure 1 and 2 supporting these results.

Discussion

To our knowledge, this is the first study to demonstrate that the *RNF213* gene polymorphism (p.R4810K) plays a role in the postoperative development of indirect, but not direct surgical collaterals after direct STA-MCA anastomosis combined with indirect pial synangiosis for adult patients with MMD. We were able to document this relationship by qualitative and quantitative evaluation of repeated TOF MR angiography during the short to mid-term follow-up period (6 to 36 months after surgery). Although evaluation of calibers of donor and recipient arteries by MR angiography rather than catheter angiography is suboptimal, the usefulness of TOF MR angiography for the evaluation of the external carotid artery tributaries after Moyamoya bypass surgery has been validated by several researchers either in a direct,[8] indirect [35] or combined bypass setting.[34] Our observation is consistent with previously published studies that favorable development of both bypass types over time is most frequent after combined direct and indirect bypass in adult MMD.[1, 33, 34]

To further determine key factors responsible for indirect and direct bypass development, respectively, we focused on significant clinical and genetic factors correlating with the excellent postoperative development of surgical collaterals by multivariate logistic regression analyses. We identified a panel of excellent revascularization-related factors for each indirect and direct bypass development after combined bypass for adult MMD (main Tables). Of note, 1) p.R4810K variant, 2) preoperative advanced MR angiographical stage, and 3) preoperative larger caliber of the MMA were positively, and 4) postoperative excellent direct bypass development was negatively correlated with excellent indirect bypass development. This is partly current knowledge. Thus, advanced angiographical stage, preoperative trans-dural collateral vessels (i.e., MMA), and heterozygous p.R4810K variant are known radiographic and genetic biomarkers of the increased capacity of postoperative surgical collateral production. [27, 29] In addition to the current knowledge, our study suggests significant correlation between p.R4810K and excellent indirect, but not direct bypass development after combined bypass, although its underlying mechanism is unclear based on our study, which is briefly discussed below. Thus, it remains unclear how p.R4810K plays a pathological role in MMD (i.e., why earlier disease onset, higher disease severity, [15, 25] and prolonged/delayed cerebral hyperperfusion after STA-MCA anastomosis [31] occur more frequently in MMD with the *RNF213* founder mutation?). Earlier experimental studies reported that cellular gene expression analysis of *RNF213* in adult human tissues revealed markedly high expression in immune tissues such as the spleen and leukocytes.[13, 23] Genome-wide plasma/serum microRNA profiling [5, 12, 32] revealed a panel of significant MMD-related plasma/serum microRNAs whose target genes were involved in inflammatory or angiogenesis-related molecular pathways. Bidirectional major pathways that are influential in the inflammatory response potentially causing collateral formation in MMD are 1) the anti-inflammatory cytokine pathway and 2) proinflammatory cytokine pathway activating *RNF213*. Fujimura et al. (2018) recently showed increased serum production of soluble CD163 and CXCL5 in MMD patients, suggesting the involvement of intrinsic M2 macrophage-related immune reactions.[7] The immune responses associated with angiogenesis are promoted by M2 macrophages and angiogenic mediators are activated through these anti-inflammatory cytokines.[23] Ohkubo et al. (2015) reported that pro-inflammatory cytokines activated transcription of *RNF213* both *in vitro* and *in vivo.* p.R4810K variant was more likely linked to the functional deficiency of the *RNF213* gene based on markedly high matrix metalloproteinase production upon experimental silencing of *RNF213*.[26] Bang et al. (2016) reported a marked increase in the blood caveolin-1 level in *RNF213* founder mutation carriers.[3] As caveolin-1 negatively regulates proliferation of endothelial cells, but positively regulates endothelial angiogenic function such as tube formation,[20] the increased caveolin-1 levels may accelerate angiogenesis in MMD patients. *In vivo* experimental study demonstrated increased angiogenesis in mice lacking *RNF213* after chronic hind-limb ischemia, [11] suggesting a role of *RNF213* abnormality in the development of pathological vascular networks in chronic ischemia. Taken together, our observation of marked angiogenesis represented by excellent indirect bypass development in adult MMD patients with P.R4810K variant can be explained bidirectionally by the loss-of-function (i.e., marked angiogenesis by lacking *RNF213* gene function) and gain-of-function (i.e., marked angiogenesis through *RNF213* mutation) mechanisms of the *RNF213* gene, which is the next question to be addressed.

Another novel finding demonstrated in this study is that the comorbid condition of dyslipidemia was negatively correlated with excellent direct bypass development. Recently, Church and Steinberg et al. (2020) reported an association between hyperlipidemia and radiological progression of unilateral type to bilateral type in MMD, with possible explanations including synergistic effects of increased lipids in the underlying moyamoya vasculopathy and the inadvertent inclusion of cerebral atherosclerotic disease in the study population.[4] One possible

explanation for our finding may also be the inadvertent involvement of atherosclerosis in our MMD patients, which leads to a poorer condition of both the donor and recipient arterial wall, resulting in poor long-term patency.

Limitation

Our study is limited by the following several points. First, our study did not include pediatric subgroup of MMD. As we previously reported that almost all (95%) pediatric MMD exhibited effective indirect revascularization after combined bypass,[33] we only investigated adult MMD in this study. Second, catheter angiography follow-up was not available in the most bypass surgeries in this study, although measurement of vessel calibers by catheter angiography is optimal. Last, consistent hemodynamic evaluations were not available for all patients using single-photon emission computed tomography or positron emission tomography. We do not consider these limitations to affect the interpretation of the results, but further studies are warranted.

Conclusions

We found that the excellent development of postoperative indirect pial synangiosis after combined direct and indirect bypass occurs more frequently in adult MMD patients with the *RNF213* founder mutation (p.R4810K) allele. This confirms a novel clinical role of the *RNF213* founder polymorphism in the marked angiogenesis via indirect pial synangiosis in adult patients with MMD, and suggests the utility of preoperative genetic analysis for *RNF213* polymorphism in MMD.

Declarations

Funding: This study was partially supported by Japanese Society for the Promotion of Science KAKENHI grant number 19H03765 (M.I.) and 20K09362 (M.F.). All authors declare that there is no personal or institutional financial interest in drugs, materials, or devices described in this article.

Conflicts of interest/Competing interests: On behalf of all authors, the corresponding author state that the authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Availability of data and material: Data supporting the findings of this study are available from the corresponding author on reasonable request.

Code availability: Not applicable

Authors' contributions: Conception and design: Fujimura M, Ito M, and Kawabori M. Acquisition of data: Ito M., Sugiyama T, Tokairin K, Tatezawa R. Analysis and interpretation of data: Ito M, Kawabori M, Sugiyama T, Tokairin K, Tatezawa R, Uchino H, and Fujimura M. Drafting the article: Ito M. Critically revising the article: Kazumata K, and Fujimura M. Statistical analysis: Ito M. Study supervision: Kazumata K and Houkin K.

Ethics approval: The present study was approved by the Hokkaido University Graduate School of Medicine medical ethics committee on human experimentation, including genetic analysis (14–053).

Consent to participate: Written informed consent was obtained from all participants (or guardians) to participate in this study.

Consent to publication: Written informed consent was obtained from all participants (or guardians) whose individual data were presented for publication in this study in any form.

Acknowledgements

We would like to acknowledge Ms. Rika Nagashima and Ms. Ikuyo Hata for their administrative assistance. We also thank Dr. Hiroyuki Kohno, Dr Kanako Hatanaka, and Ms. Miyuki Kasegai for their technical support in the genetic analysis.

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Tables

TABLE 1 Correlation of Postoperative Indirect bypass development with Clinical and Genetic Variables

		Indirect bypass development		Unadjusted		Adjusted	
Variables		Excellent	Non-excellent	Odds Ratio (95% CI)	*P* value	Odds Ratio (95% CI)	*P* value
		(n = 65)	(n = 28)				
RNF213 founder polymorphism, A/G		46/65 (71%)	13/28 (46%)	2.8 (1.1 to 7.1)	0.028	**4.0 (1.2 to 16)**	**0.034**
Age, y, mean, SD		41 ± 11	43 ± 9.5	0.98 (0.94 to 1.0)	0.46		
Sex, Male		13/65 (20%)	8/28 (29%)	0.63 (0.23 to 1.8)	0.37		
Familial occurrence		23/65 (35%)	7/28 (25%)	1.6 (0.63 to 4.7)	0.33		
Hemisphere, Left		33/65(51%)	12/28 (43%)	1.4 (0.57 to 3.4)	0.48		
Clinical presentation	Ischemia	45/65 (69%)	17/28 (61%)	1.5 (0.57 to 3.7)	0.43		
	Hemorrhage	5/65 (7.7%)	5/28 (18%)	0.38 (0.10 to 1.5)	0.16		
MR angiographical stage	2 (reference)	12/65 (18%)	18/28 (64%)	-	-	-	-
	3	34/65 (52%)	7/28 (25%)	7.3 (2.5 to 23)	0.0004	**13 (3.4 to 62)**	**0.0004**
	4	19/65 (29%)	3/28 (11%)	9.5 (2.6 to 47)	0.0019	**9.5 (1.7 to 73)**	**0.017**
PCA involvement		17/65 (26%)	1/28(3.6%)	9.6 (1.8 to 177)	0.033	4.1 (0.48 to 92)	0.25
Co-morbidities	Hypertension	20/65 (31%)	10/28 (36%)	0.80 (0.32 to 2.1)	0.64		
	Diabetes mellitus	4/65 (6.3%)	2/28 (7.1%)	0.85 (0.16 to 6.4)	0.86		
	Dyslipidemia	12/65 (19%)	1/28 (3.6%)	6.1 (1.1 to 114)	0.09	12 (1.3 to 278)	0.052
Decreased cerebrovascular reserve		24/40 (60%)	9/17 (53%)	1.3 (0.42 to 4.2)	0.62		
(10% or less)							

Direct bypass development, Excellent		40/65 (62%)	25/28 (89%)	0.19 (0.043 to 0.62)	0.013	**0.17 (0.03 to 0.75)**	**0.029**
Preoperative donor artery diameter							
mm, mean, SD	STA	1.8 ± 0.36	1.9 ± 0.32	0.83 (0.23 to 3.0)	0.78		
	MMA	1.6 ± 0.45	1.4 ± 0.40	3.9 (1.3 to 14)	0.023	**6.8 (1.8 to 35)**	**0.010**
	DTA	1.0 ± 0.36	0.95 ± 0.34	1.6 (0.41 to 6.7)	0.50		
	BA	3.0 ± 0.47	3.1 ± 0.61	0.67 (0.27 to 1.6)	0.36		
Days after bypass for the assessment		305 ± 127	350 ± 167	1.0 (1.0 to 1.0)	0.17		

Abbreviations: RNF213, ring finger protein 213; CI, confidence interval; SD, standard deviation; PCA, posterior cerebral artery; STA, superficial temporal artery; MMA, middle meningeal artery; DTA, deep temporal artery; BA, basilar artery

TABLE 2 Correlation of Postoperative Direct bypass development with Clinical and Genetic Variables

		Direct bypass development		Unadjusted		Adjusted	
Variables		Excellent	Non-excellent	Odds Ratio (95% CI)	*P* value	Odds Ratio (95% CI)	*P* value
		(n = 65)	(n = 28)				
RNF213 founder polymorphism, A/G		41/65 (63%)	18/28 (64%)	0.95 (0.37 to 2.4)	0.91		
Age, y, mean, SD		42 ± 9.9	42 ± 11	1.0 (0.96 to 1.0)	0.93		
Sex, Male		15/65 (23%)	6/28 (21%)	1.1 (0.39 to 3.4)	0.86		
Familial occurrence		21/65 (32%)	9/28 (32%)	1.0 (0.40 to 2.7)	0.99		
Hemisphere, Left		33/65 (51%)	12/28 (43%)	1.4 (0.57 to 3.4)	0.48		
Clinical presentation	Ischemia	42/65 (65%)	20/28 (71%)	0.71 (0.26 to 1.8)	0.52		
	Hemorrhage	7/65 (11%)	3/28 (11%)	1.0 (0.26 to 5.0)	0.99		
MR angiographical stage	2 (reference)	23/65 (35%)	7/28 (25%)	-	-		
	3	28/65 (43%)	13/28 (46%)	0.66 (0.22 to 1.9)	0.44		
	4	14/65 (22%)	8/28 (29%)	0.53 (0.15 to 1.8)	0.31		
PCA involvement		11/65 (17%)	7/28(25%)	0.61 (0.21 to 1.9)	0.37		
Co-morbidities	Hypertension	21/65 (32%)	9/28 (32%)	1.0 (0.40 to 2.7)	0.99		
	Diabetes mellitus	3/65 (4.6%)	3/28 (11%)	0.40 (0.07 to 2.3)	0.29		
	Dyslipidemia	5/65 (7.7%)	8/28 (29%)	0.21 (0.057 to 0.70)	0.012	**0.27 (0.073 to 0.93)**	**0.042**
Decreased cerebrovascular reserve		20/39 (51%)	13/18 (72%)	0.40 (0.11 to 1.3)	0.14		
(10% or less)							
Indirect bypass development, Excellent		40/65 (62%)	25/28 (89%)	0.19 (0.043 to 0.62)	0.013	**0.23 (0.05 to 0.77)**	**0.029**
Preoperative donor artery							

diameter					
mm, mean, SD	STA	1.8 ± 0.32	1.9 ± 0.36	1.2 (0.35 to 4.6)	0.75
	MMA	1.5 ± 0.32	1.6 ± 0.49	1.9 (0.67 to 5.7)	0.24
	DTA	0.98 ± 0.30	0.98 ± 0.34	1.1 (0.27 to 4.2)	0.94
	BA	3.1 ± 0.51	3.0 ± 0.52	0.79 (0.33 to 1.9)	0.60
Days after bypass for the assessment		307 ± 105	323 ± 154	1.0 (1.0 to 1.0)	0.61

Abbreviations: RNF213, ring finger protein 213; CI, confidence interval; SD, standard deviation; PCA, posterior cerebral artery;

STA, superficial temporal artery; MMA, middle meningeal artery; DTA, deep temporal artery; BA, basilar artery

Figures

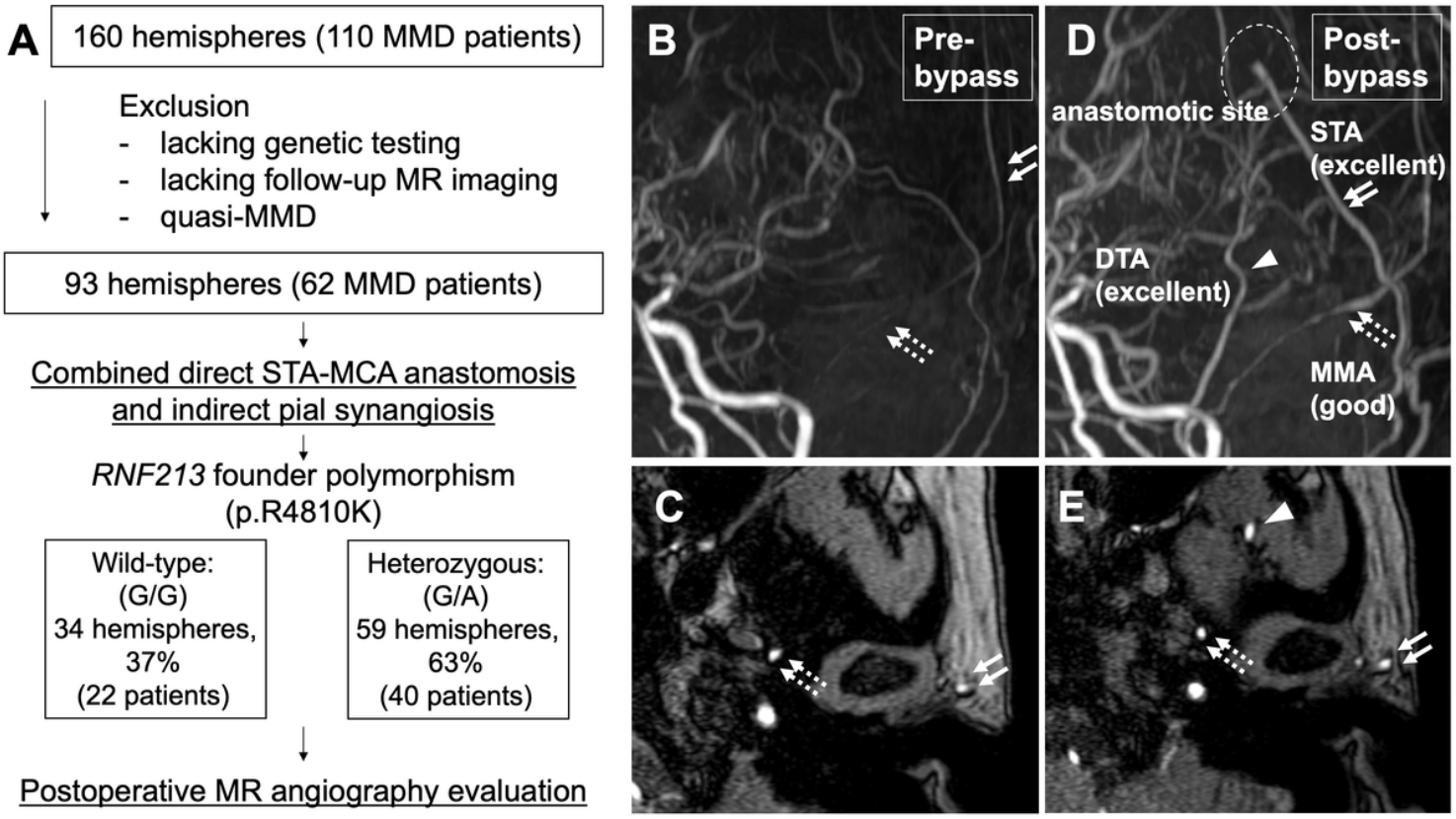

Figure 1

Flow chart for inclusion of the study subjects and representative MR angiography of a study subject before and after combined direct/indirect bypass (A) Flow chart shows the breakdown of inclusion and exclusion of the study subjects. (B-E) MR angiography of a 49-year-old female with Moyamoya disease obtained before and 6

months after left combined direct and indirect bypass. Preoperative axial time-of-flight MR angiography with maximal intensity projection (MIP) reconstruction (B) and its source image (C) of a 49-year-old MMD female with the RNF213 founder polymorphism (A/G genotype) showed the left superficial temporal artery (STA, double arrows) and middle meningeal artery (MMA, dotted arrows). The deep temporal artery was hardly seen in MIP or its source images preoperatively. (D, E) Follow-up MR angiography obtained 6 months after left STA-MCA anastomosis combined with encephalo-duro-myo-arterio-pericranio-synangiosis demonstrated excellent development of postoperative direct (STA) and indirect (MMA and DTA) surgical collaterals.

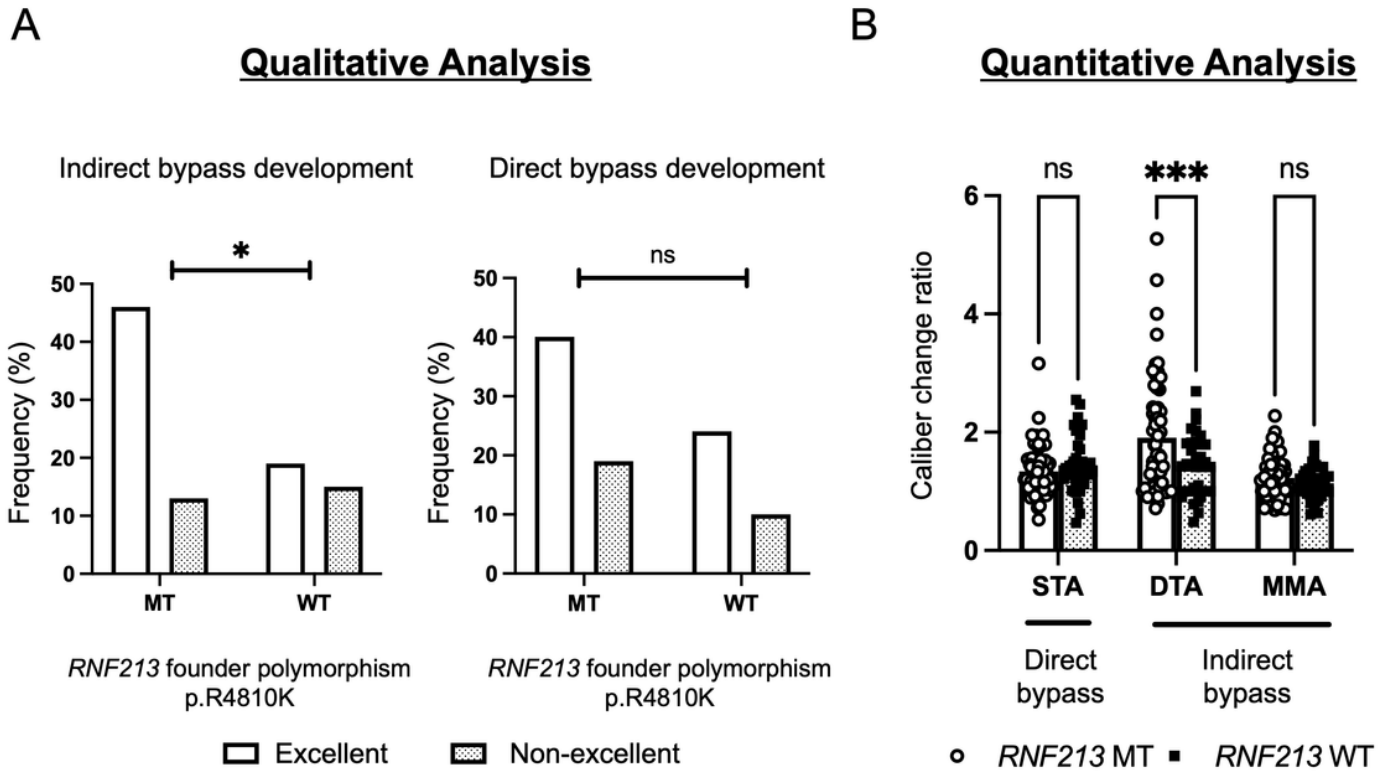

Figure 2

Qualitative and quantitative analysis of postoperative direct and indirect collateral development using time-of-flight magnetic resonance angiography (A) Interleaved bar graph showing the results of qualitative analysis of the axial time-of-flight MR angiography with maximal intensity projection (MIP) reconstruction before and after combined bypass to evaluate the development of postoperative indirect and direct collaterals in adult MMD. ns, not significant; *P <0.05, Fisher's exact test. (B) Interleaved scatterplot with bars showing the caliber change ratios for the superficial temporary artery (STA), deep temporal artery (DTA), and middle meningeal artery (MMA) from quantitative analysis of the source images of MR angiography before and after combined bypass in adult MMD. ns, not significant; ***P <0.001, two-way ANOVA followed by Bonferroni multiple comparison.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppleTABLE1.pdf

- SuppleTABLE2.pdf
- Supplementaryfile.docx
- supplfigure1.pdf
- supplfigure2.pdf