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Impact of *RNF213* p.R4810K variant on Postoperative Temporal Muscle Swelling Used in Encephalo-Myo-Synangiosis After Combined Revascularization for Moyamoya Disease

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Abstract

Postoperative neurological deterioration due to brain compression by the swollen temporal muscle pedicle used in encephalo-myo-synangiosis (EMS) is a potential complication of combined revascularization for Moyamoya disease (MMD). However, the factors contributing to this phenomenon remain poorly understood. This study aimed to identify factors associated with postoperative temporal muscle swelling following combined revascularization. A total of 37 consecutive combined revascularization using temporal muscle pedicle performed between 2021 and 2023 were analyzed. Postoperative temporal muscle volume was measured through serial CT scans on postoperative days (POD) 0, 1, 7, 14, and 30. Multiple regression analysis was performed to assess factors contributing to swelling, including *RNF213* p.R4810K variant, a known genetic risk for Asian MMD. Surgical outcomes and collateral vessel development were also examined. Results showed a significant increase in muscle pedicle volume on POD 1 and 7 across all 37 hemispheres, followed by a marked decrease by POD 30, compared to POD 0. These chronological volume changes were significant in adults (n=31) but not in pediatric patients (n=6). Multiple regression analysis identified the *RNF213* p.R4810K as sole significant factor positively associated with maximal muscle volume (regression coefficient 0.485, P=0.0078). Favorable surgical outcomes were achieved in 36 of 37 cases (97.3%) over a mean follow-up of 2.2 years, with indirect collateral development confirmed in 27 adult (87%) and 6 pediatric (100%) hemispheres. Results suggest the *RNF213* p.R4810K variant is associated with increased postoperative temporal muscle swelling after combined revascularization, especially in adult MMD patients, indicating a potential genetic influence on this complication.

Keywords: Encephalo-myo-synangiosis, Moyamoya disease, Revascularization, *RNF213* variant, Surgical complication, Temporal muscle swelling

Introduction

Moyamoya disease (MMD) is a rare cerebrovascular disorder characterized by chronic/progressive stenosis at the terminus of the intracranial internal carotid artery, accompanied by the development of abnormal fragile vascular networks [1]. This condition leads to ischemic and/or hemorrhagic stroke predominantly in pediatric or young adult population. To date, a strong association has been identified with *RNF213* genetic variant (p.R4810K) in Japanese, Korean, and Chinese patients [2-5]. Combined direct (i.e., superficial temporal artery-middle cerebral artery; STA-MCA anastomosis) and indirect bypass (i.e., encephalo-duro-myo-synangiosis; EDMS or encephalo-myo-synangiosis; EMS), is widely accepted as a procedure for preventing future stroke in adult patients with MMD [6-7].

The indirect bypass procedure involving the use of temporal muscle necessitates the intracranial insertion of the muscle pedicle under the cranial bone flap to cover a large area of the brain [8-10]. Postoperative neurological deterioration caused by local hypoperfusion due to brain compression by the swollen temporal muscle pedicle used for EMS, is a rare but potential complication after combined revascularization in MMD [11-15]. Recently, we revealed the incidence of transient neurological events due to the swollen temporal muscle was 5.6% (one out of 18 consecutive procedures) [14]. Consequently, in our previous study cohort, the intracranially inserted temporal muscle pedicle volume increased by as much as 1.12 by postoperative day (POD) 7, relative to POD0. Despite the substantially standardized surgical procedure, the degree of postoperative temporal muscle swelling varied in our previous study, and the contributing factors remain undetermined [14]. Therefore, this study aimed to investigate the factors contributing to postoperative temporal muscle swelling after combined revascularization using temporal muscle pedicle in MMD. To uncover the mechanism underlying postoperative temporal muscle pedicle swelling and the role of genetic variant, *RNF213* p.R4810K on this phenomenon, we would discuss the link between these two seemingly unrelated manifestations based on the concurrent knowledge from the literature.

Methods

Patients

In this prospective observational cohort, we included a total of 37 combined revascularization procedures in 32 consecutive Japanese MMD patients. All surgeries were primarily operated by a single neurosurgeon (M.F.) at our institution between January 2021 and January 2023. Of these, eighteen procedures in 15 patients were included in our previous analysis [14]. All patients were symptomatic as presented with ischemic or hemorrhagic stroke symptoms but showed independent performance status at surgery. The diagnosis of MMD and surgical indication

were determined based on the cerebral angiography (except for young pediatric cases) according to the current Japanese guidelines for the diagnosis and management of MMD [2, 16]. In this study, we defined adult MMD when patients were 16 years old or older at surgery as defined elsewhere [17].

Surgical procedure

Our combined revascularization was standardized as a direct STA-MCA (cortical M4 branch) single anastomosis combined with EDMS using temporal muscle with meticulous perioperative critical care. Our standardized surgical procedure and perioperative critical care from early-stage post-surgery was documented in detail in our previous report [14,18,19]. Briefly, an L-shaped skin incision was made along with the frontal or parietal branch of the STA, followed by gentle dissection of the temporal muscle by a monopolar electrode between the muscle and bone. The deep temporal artery (DTA) and deep temporal vein were preserved to minimize postoperative temporal muscle swelling. An inverted triangle-shaped craniotomy was performed with gentle retraction of the temporal muscle. In an end-to-side manner, STA-MCA anastomosis was performed using 10-0 monofilament suture. The patency was confirmed by intraoperative indocyanine green videoangiography and Doppler ultrasonography. To prevent postoperative complication due to temporal muscle swelling, we routinely performed EDMS with the following attempts: i) drilling the inner layer of the bone flap for insurance against transient temporal muscle swelling, ii) creating a cranial bone window wide enough for pedicle insertion to avoid strangulation of the swollen temporal muscle pedicle, and iii) splitting the temporal muscle into two layers to reduce its thickness if applicable. After cranioplasty, the remaining outer layer of the muscle was placed over the bone flap, followed by skin closure without the placement of a drainage tube.

Genetic Analysis

For the genetic testing, peripheral blood samples were collected from all the participants before surgery. In accordance with the institutional review board-approved protocol, genetic analysis was conducted at research division of genome companion diagnostics in our hospital who were blinded to individual clinical data (Supplement). Briefly, total DNA was extracted from the peripheral blood, and Taqman single nucleotide polymorphism genotyping assay (TaqMan SNP Genotyping Assay, Catalog number 4351379, Thermo Fisher Sci. MA, USA) was employed to determine the allele type for *RNF213* p.R4810K polymorphism, a known genetic risk for Asian individuals with MMD [3,4].

Time-course analysis of the intracranial temporal muscle pedicle volume

Postoperative temporal muscle pedicle volume was evaluated using 5-mm-slice computed tomography (CT) scans acquired on POD0, 1, 7, 14, and 30. We employed the XYZ/2 technique to provide the total volume of the inserted

muscle pedicle as in our previous report [14, 20]. Briefly, the maximum thickness, length, and height of the intracranially inserted temporal muscle pedicle were determined on appropriate axial CT images (5-mm-thickness) on which the temporal muscle pedicle was visible. For the maximal height measurement (Z axis for CT scans), the number of slices visible for the temporal muscle pedicle was counted and multiplied by 5-mm (thick ness of each axial slice, please see the diagram in the Supplementary figure). This was validated by coronal CT images to confirm its accuracy (data not shown). The volume calculated using the XYZ/2 method was previously validated against computer-assisted volumetric assessment, which demonstrated a significant correlation (please refer to supplementary results for further details). These measurements were performed by first author (M.M) blinded to the results of genetic testing and latest surgical outcome. For the time-course analysis of volume changes after surgery, the relative temporal muscle volume at each postoperative time point was calculated relative to that on POD0.

Surgical outcome

Postoperative development of both direct and indirect bypasses was qualitatively evaluated using time-of-flight (TOF) magnetic resonance angiography (MRA). These evaluations were conducted in outpatient settings later than six months post-surgery following a previously reported protocol [21-23]. Briefly, postoperative development of each direct and indirect surgical collaterals was dichotomized (i.e., good or poor). The development of direct surgical collaterals was evaluated by the visibility of the STA, while that of indirect surgical collaterals was evaluated by that of middle meningeal artery (MMA) and/or DTA. We evaluated surgical outcome as the recurrence of stroke or transient ischemic attack (TIA) and intracranial hemorrhage (ICH), as well as the modified Rankin Scale (mRS) at the latest clinic visit or interview by phone call (mean follow-up period: 2.2±0.6 years post-surgery).

Statistical analysis

Continuous variables (i.e., age and the relative temporal muscle volume) were expressed as mean and standard deviation and rank/categorical variables (i.e., sex, hemisphere side) was expressed as ratios or frequency. For multiple comparisons of the relative temporal muscle volume at each postoperative time point with that at POD0, we used fitting-a-mixed-effects model to analyze repeated measures with missing values followed by the post-hoc Dunnett test. Nine values (CT on POD7 in 5 patients/procedures, on POD14 in 3 patients/procedures, and POD30 in 1 patient/procedure) were missing because MR scans were performed instead of CT scans for random reasons. To identify factors potentially contributing to the postoperative temporal muscle volume changes, mean maximal relative temporal muscle pedicle volume was analyzed in relation to each demographic and baseline clinical

variables across all 37 hemispheres by univariate linear regression or unpaired t-test or Mann-Whiteney U-test for continuous or rank/categorical variable, respectively. Subsequently, forced entry multiple linear regression test was performed. We included following variables, including age at surgery; sex; hemispheric side of revascularization; clinical presentation, i.e., transient ischemic attack, cerebral infarct, and intracerebral hemorrhage; Suzuki's angiographical stage; comorbidities, i.e., diabetes mellitus, hypertension, dyslipidemia; preoperative systemic immune inflammation index, SII; *RNF213* p.R4810K variant type; preoperative antiplatelet agent, i.e., aspirin, cilostazol; operation time, and cranial bone window area as the potential factors contributing to the postoperative temporal muscle volume changes. The SII was calculated based on the blood cell counts collected before surgery using the following formula: platelet count (number*10^3/uL)*absolute neutrophil count (/uL)/absolute lymphocyte count (uL) as reported elsewhere [24,25]. The level of significance was set at $P < 0.05$. GraphPad Prism (version 10.0.3, San Diego, CA, USA) was used to conduct all these analyses.

Results

Patient demographic, genetic, clinical features

This study included 31 hemispheres in 27 adult patients (ranging from 17 to 60 years old) and 6 hemispheres in 5 pediatric patients (ranging from 8 to 14 years old). The demographic, genetic and clinical features were summarized in Table 1. Our combined revascularization in this prospective cohort was predominantly STA-MCA single anastomosis with indirect EDMS procedure in a mean operation time of 245±35 minutes. In all the 37 procedures, the patency of STA-MCA bypass was confirmed by intraoperative indocyanine green videoangiography and Doppler ultrasonography. In addition, all the indirect EDMS bypasses were achieved using vascularized temporal muscle and dural pedicles.

Chronological volume changes of the temporal muscle pedicle after combined revascularization in patients with MMD

The mean values of absolute temporal muscle pedicle volume evaluated by the XYZ/2 method were as follows (n=37, mean±standard deviation): 10.4±3.6 cm3 on POD 0, 12.7±5.0 cm3 on POD 1, 13.6±6.1 cm3 on POD 7, 10.7±4.4 cm3 on POD 14, and 6.2±3.5 cm3 on POD 30 (Figure5 and Supplementary figure). The relative volume (relative to that of POD0) of the intracranial temporal muscle pedicle was 1.23±0.29 on POD1, 1.33±0.38 on POD7, 1.06±0.31% on POD14, and 0.59±0.23 on POD30. As shown in Figure 1, the relative volume significantly increased on POD1 and 7, then significantly decreased by POD30 across all the 37 hemispheres. Thus, Figure 1 depicted chronological volume increase of the temporal muscle pedicle by POD7 and decrease by POD30 after

the combined revascularization. To evaluate the effect of patient age on the chronological volume changes, we conducted a time-course analysis in both adult (older than 16 years of age at surgery) and pediatric patient groups (younger than 15 years of age), respectively (Figure 2). In the adult group, a significant increase in relative muscle volume on POD1 and 7, as well as a significant decrease on POD30 were observed, respectively. In contrast, the pediatric group showed no significant differences in relative muscle volume on POD1, 7, and 14, while a significant decrease was observed on POD30. Taken together, the time-course analysis suggested postoperative temporal muscle swelling occurred on POD1 and 7 after combined revascularization, particularly in adult patients with MMD. By POD30, the inserted temporal muscle pedicle gradually involuted in both age groups.

Multiple linear regression analysis for maximal relative temporal muscle volume

To investigate the factors contributing to the postoperative temporal muscle swelling, maximal relative temporal muscle pedicle volume was analyzed in relation to each demographic and baseline clinical variables across all 37 hemispheres by univariate analyses (Table 2). Subsequent forced entry multiple linear regression analysis revealed *RNF213* p.R4810K variant (G/A) as sole significant factor positively correlated with the postoperative maximal relative volume (regression coefficient, 0.485; 95% confidence interval, 0.145 to 0.826, P=0.0078, Table 2). Other factors, including demographic, preoperative clinical and surgical factors were not significantly correlated with postoperative maximal relative volume. In fact, the maximal relative volume was significantly higher in patients with *RNF213* p.R4810K variant than those without (1.4±0.30 and 1.1±0.19, respectively P=0.0041, Figure 3A). As shown in Figure 3B, chronological increase of the relative volume was significant on POD1 and 7 when compared to that of POD0 in MMD with *RNF213* p.R4810K variant (G/A) but not in wild-type (G/G), while significant decrease was observed by POD30 regardless of *RNF213* p.R4810K variant type. These results were visually confirmed by scatter plot and linear regression analysis result as shown in Figure 4. Together, *RNF213* p.R4810K variant (G/A) may predispose patients to postoperative temporal muscle swelling after combined revascularization, particularly in adult MMD. Regardless of the *RNF213* p.R4810K variant type, the inserted temporal muscle pedicle involuted by POD30.

Surgical outcome

Out of 37 consecutive combined revascularizations in this prospective cohort, we observed one case with postoperative cerebral ischemia due to compression of the brain by swollen temporal muscle (2.7%). Postoperative symptomatic focal cerebral hyperperfusion was observed in one different case (2.7%). Therefore, the incidence of overall perioperative surgical complication was 5.4% in this prospective cohort observed within two weeks after surgery. During a mean follow-up period of 2.2±0.6 years post-surgery, two patients experienced ipsilateral

recurrence of stroke (5.4%), including cerebral infarct in one pediatric patient and repeated intraventricular hemorrhage at the remote area from the direct anastomosis in one adult patient. Except for one unfavorable case (mRS of 5), most of the cases showed independent favorable outcome (mRS 0-2) during the follow-up period. Postoperative collateral development via direct and indirect bypass was confirmed later than six months after surgery (mean follow-up period: 10.3±3.1 months post-surgery) in 30 (97%) and 21 (68%) hemispheres in adult cases, respectively (Table 3). Whereas all the pediatric cases showed postoperative collateral development via both direct and indirect bypass. Thus, postoperative direct bypass development was observed in all the 37 cases regardless of the *RNF213* p.R4810K variant type, except for one adult case with the *RNF213* p.R4810K wild-type (G/G). On the other hand, indirect bypass development was observed in 21(77.8%) out of 27 *RNF213* p.R4810K variant (G/A) and 6 (60%) out of 10 *RNF213* p.R4810K wild-type (G/G). There was no correlation between the *RNF213* p.R4810K variant type and postoperative direct/indirect bypass development. We addressed whether postoperative temporal muscle swelling is associated with the surgical collateral development, particularly with indirect bypass development in the adult group. As a result, maximal relative temporal muscle volume was not significantly different between patients with good and poor indirect bypass development in adult MMD (1.3±0.35 and 1.3±0.27, P=0.71). These results suggested combined revascularization using temporal muscle pedicle in this prospective cohort provided favorable surgical outcomes in both adult and pediatric MMD patients. These results were achieved when the aforementioned surgical attempts and meticulous perioperative care were applied according to current diagnostic criteria and management guidelines [2,16].

Representative cases

A 54-year-old man presenting with cerebral infarct due to MMD (Suzuki's angiographical stage 3) underwent combined revascularization, including EDMS with the aforementioned surgical attempts on his left hemisphere. The relative muscle volume was increased at 1.65 on POD1 and 2.00 on POD7 relative to POD0 (Figure 5A, lower panels). With the use of Chinese KANPO medicine (i.e., JIDABOKUIPPO), his postoperative course was uneventful and the relative volume was decreased at 0.99 by POD30. As shown in Figure 5A (upper panels), MR angiography on POD 2 showed patent direct STA-MCA bypass. MR angiography after eight months of surgery depicted patent direct STA-MCA bypass and indirect bypass development (i.e, DTA). Genetic testing revealed he was *RNF213* p.R4810K heterozygote (G/A). By the latest clinic visit at 1.7 years after surgery, he did not experience any stroke recurrence with independent daily life (mRS of 0). In contrast, a 57-year-old man with transient motor weakness on his right due to ischemic-onset MMD (Suzuki's angiographical stage 3) underwent the combined revascularization on his left hemisphere. The relative muscle volume was increased at 1.31 on POD1

and 1.33 on POD7 relative to POD0 (Figure 5B, lower panels). As shown in Figure 5B (upper panels), MR angiography after 12 months of surgery showed both direct (STA) and indirect bypass (MMA) development. Genetic testing revealed he was wild-type for the *RNF213* p.R4810K variant (G/G). By the latest clinic visit at 1.6 years after surgery, he did not experience any stroke recurrence with independent daily life (mRS of 0).

Discussion

In this single-institution prospective study, significant temporal muscle swelling, used for EDMS, was observed on POD1 and 7 following combined revascularization in MMD patients with the *RNF213* p.R4810K heterozygosity. Subsequently, the inserted temporal muscle pedicle significantly involuted by POD30, regardless of the *RNF213* p.R4810K variant type. While the time-course analysis for the postoperative temporal muscle volume demonstrated significant increase on POD1 and 7 after combined revascularization, particularly in adult patients with MMD. By POD30, the inserted temporal muscle pedicle gradually involuted in both age groups. In this prospective cohort, combined revascularization using temporal muscle pedicle provided favorable surgical outcomes in both adult and pediatric MMD patients when the aforementioned surgical attempts and meticulous perioperative care were applied. Based on these observations, the adult MMD patients with the *RNF213* p.R4810K variant (G/A) may have a higher risk of postoperative temporal muscle swelling after combined revascularization using temporal muscle but could achieve favorable functional outcomes. This is the first report demonstrating a significant correlation between the *RNF213* p.R4810K variant and temporal muscle pedicle swelling after combined revascularization in MMD.

In this study, we were unable to uncover the exact mechanism underlying the predisposition of *RNF213* p.R4810K heterozygote patients (G/A) to postoperative temporal muscle pedicle swelling after the combined revascularization. Growing evidence supports the genotype-phenotype correlation of the *RNF213* p.R4810K variant following the identification of the *RNF213* gene as a susceptibility gene for MMD across diverse populations [3-5]. Certain MMD phenotypes associated with the *RNF213* p.R4810K include baseline clinical manifestations (e.g., early onset, cerebral infarct at presentation) [26,27], pre-operative angiographical features [28-30] (e.g., involvement of the posterior cerebral artery, progression of unilateral MMD to contralateral hemisphere, and spontaneous collateral blood supply from external carotid system), as well as post-revascularization surgical complication [31,32] and surgical collateral vessel development [22,23,33-35]. Torazawa et al. recently demonstrated a significant positive correlation between the *RNF213* p.R4810K variant and the caliber change ratio (CCR) of DTA after direct STA-MCA anastomosis with EMS in the chronic phase,

between 6 months and 1 year after surgery [33]. However, they did not study postoperative temporal muscle swelling in the acute phase following combined revascularization. By integrating the results of previous studies—specifically, the significant in-growth of DTA-derived indirect collateral via the temporal muscle pedicle (EMS) in the p.R4810K heterozygotes—and the findings of our study (the predisposition of p.R4810K heterozygotes to postoperative temporal muscle swelling), it can be inferred that *RNF213* p.R4810K variant might have a role in post-revascularization changes of the temporal muscle pedicle in MMD. A recent review summarized the basic functions of the *RNF213* gene, which plays a key role in lipid metabolism, oxygen consumption, cell cycle control, and NF-κB-mediated inflammation, contributing to vascular cell maintenance [36]. MMD-associated mutations in the RING domain enhance NF-κB activation, inducing inflammation [4,36,37]. Thus, our findings can offer new insights into the impact of the *RNF213* p.R4810K variant on the post-revascularization response of the temporal muscle pedicle in the acute (temporal muscle swelling) and chronic phase (in-growth of DTA in the temporal muscle) via altered immune/inflammatory response by *RNF213* p.R4810K variant in MMD. **Although this study did not directly establish a significant association between muscle swelling and the development of indirect bypass, we plan to address the relationship between postoperative muscle swelling and indirect bypass development in larger and more diverse cohorts.**

Based on the current results, we might be able to identify adult MMD patients at risk of postoperative muscle swelling after combined revascularization, which effectively prevent future stoke events. Thus, recent systematic reviews and meta-analyses have suggested that combined direct and indirect revascularization is favored over indirect bypass surgery in adult MMD for favorable outcomes [6,7]. In pediatric patients, indirect or combined revascularization has been shown to improve both short- and long-term clinical outcomes [9,10,38-41]. Surgical revascularization is generally considered reasonable for MMD presenting with ischemic or hemorrhagic symptoms, particularly posterior hemorrhage [17,42]. However, postoperative cerebral hyperperfusion syndrome in adults [19,43] and perioperative cerebral infarction or global hypoperfusion in pediatric patients remain concerns [44,45]. Perioperative management, including mild blood pressure control and the use of neuroprotective/anti-inflammatory agents [46,47], alongside efforts to mitigate postoperative temporal muscle swelling [12-14,48], are essential to achieve favorable outcomes. Symptomatic brain compression due to swollen temporal muscle is reported in 5.5% to 6.2% of adult cases [12,14] and up to 16.2% in pediatric case series [13]. Based on our previous study [14], the risk factors contributing to postoperative temporal muscle swelling remained obscure, except for mechanical temporal muscle injury and venous congestion of the muscle pedicle. In this prospective cohort, we could demonstrate adult MMD patients with *RNF213* p.R4810K variant (G/A) have a higher risk of postoperative

temporal muscle swelling compared to those without (i.e., *RNF213* p.R4810K wild-type, G/G). Given that the hemodynamically impaired brain in MMD is vulnerable to increased intracranial pressure, mechanical compression by the swollen temporal muscle could cause focal cerebral ischemia, leading to neurological deterioration [11-14]. Regarding multiple choice of indirect procedures (i.e., EAS, EDS, EMS, EDMS, EDAMS, EDMAPS, and omental-cranial transposition etc.), each has each advantage and disadvantage. Since a pedicled vascularized tissue- such as temporal muscle pedicle or omental flap [49] is highly vascularized compared with dura mater, we favored the STA-MCA anastomosis combined with indirect procedure using temporal muscle pedicle as our standard procedure both for adults and pediatric patients if applicable [18,44]. Based on our current and previous studies, indirect bypass using temporal muscle pedicle has disadvantage such as postoperative temporal muscle swelling causing ischemic complication [11-14] but has advantage, such as better collateral in-growth through the temporal muscle (i.e., deep temporal artery) in MMD patients with RNF213 p.R4810K variant [22,23]. Based on these considerations, among the various indirect procedures, we favored to use temporal muscle pedicle as an in-situ harvestable and highly vascularized tissue for better post-operative collateral formation. Altogether, genotyping for the *RNF213* p.R4810K variant may contribute to further risk stratification in combined revascularization using temporal muscle pedicle in adult MMD. In future clinical practice, precision medicine approaches—such as elective combined revascularization using temporal muscle pedicle tailored to genetically selected patients—may contribute to improved prognoses for individuals with MMD.

This study has several limitations. First, our study population consisted of a small sample size from single institute, particularly in pediatric patients. **We are committed to further validating our observations and enhancing the generalizability of our findings in larger and more diverse multi-center cohorts.** Second, the follow-up period for surgical outcomes was relatively short. In fact, all pediatric patients who participated in this study were *RNF213* p.R4810K heterozygote (G/A). This may underestimate the impact of the *RNF213* p.R4810K variant on postoperative temporal muscle swelling in pediatric MMD patients. Third, while we standardized the surgical procedure to prevent postoperative temporal muscle swelling, we did not have a prevention protocol specific for MMD patients with the *RNF213* p.R4810K variant. A more precise understanding of MMD is essential, including molecular functions of *RNF213* gene and associated genetic variants, to develop new treatment strategies.

Conclusions

Adult MMD patients with the *RNF213* p.R4810K variant may have a higher risk of postoperative temporal muscle swelling after combined revascularization, suggesting a predisposition of the genetic variant to transient

postoperative swelling. Although the underlying mechanisms remain unclear, our findings provide new insights into the role of *RNF213* p.R4810K in influencing the clinical phenotype of MMD. To achieve favorable surgical outcomes, it is crucial to mitigate the adverse effects of postoperative temporal muscle swelling, particularly in adult MMD patients with the *RNF213* p.R4810K variant.

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Figure Legend

Figure 1: Chronological volume changes of the temporal muscle pedicle following combined revascularization across all the 37 hemispheres of Moyamoya disease (MMD)

An interleaved scatter plot with bars illustrates chronological changes of temporal muscle pedicle volume at Postoperative Days (PODs)1, 7, 14, and 30 following combined revascularization, including encephalo-duro-myo-synangiosis. The values were normalized to the volume on POD0.

Error bars express standard error of means. **: P< 0.01, ****: P < 0.0001, ns: not significant.

Figure 2: A time-course analysis of the temporal muscle pedicle volume changes following combined revascularization in both adult and pediatric patient groups

Interleaved scatter plots with bars illustrates the relative temporal muscle pedicle volume relative to Postoperative Day (POD)0 at POD1, 7, 14, and 30 following the combined revascularization in adult (A) and pediatric patients with MMD (B).

Error bars express standard error of means. **:P<0.01, ****:P < 0.0001, ns: not significant.

Figure 3: Impact of *RNF213* p.R4810K on chronological volume changes following combined revascularization in Moyamoya disease (MMD)

(A) An interleaved scatter plot with bars illustrates the significant difference in maximal relative temporal muscle volume between *RNF213* variant and wild-type.

(B) A time-course analysis of the temporal muscle pedicle volume changes following combined revascularization in MMD patients with *RNF213* variant (G/A) and wild-type (G/G).

Error bars express standard error of means. **:P<0.01, ***:P < 0.001, ****:P < 0.0001

Figure 4: Scatter plot and linear regression analysis for age at surgery and genotypes of *RNF213* p.R4810K

A scatter plot and regression analysis result for the age and *RNF213* p.R4810K factors showing the visual relationship with the maximal relative temporal muscle volume following combined revascularization with encephalo-duro-myo-synangiosis in patients with Moyamoya disease. Red dots represent the cases with G/A (p.R4810K heterozygote), while blue dots indicate those with G/G (p.R4810K wild-type).

Figure 5: Representative cases with postoperative temporal muscle pedicle volume changes after combined revascularization in adult Moyamoya disease (MMD) patients with *RNF213* variant (G/A) and wild-type (G/G).

(A) A 54-year-old man with infarct-onset MMD and *RNF213* p.R4810K variant (G/A) underwent left-sided combined revascularization, including encephalo-duro-myo-synangiosis (EDMS). His temporal muscle pedicle volume increased 1.65 times on Postoperative Day (POD)1 and 2.00 times on POD7 (white arrow in the lower panels) relative to POD0. Magnetic Resonance (MR) angiography on POD2 shows excellent patency of the superficial temporal artery to middle cerebral artery (STA-MCA) bypass (white arrows in the upper panels). MR angiography after eight months (8M) revealed good collateral development via middle meningeal artery and deep temporal artery (arrow heads in the upper panels).

(B) A 57-year-old man with transient ischemic attack (TIA)-onset MMD and *RNF213* p.R4810K wild-type (G/G) underwent left-sided combined revascularization, including EDMS. His temporal muscle pedicle volume increased 1.31 times on POD1 and 1.33 times on POD7 (white arrow in the lower panels) relative to POD0. MR angiography shows excellent direct bypass development (white arrows in the upper panels) and good indirect bypass development via middle meningeal artery (arrow heads in the upper panels).

Figure 1

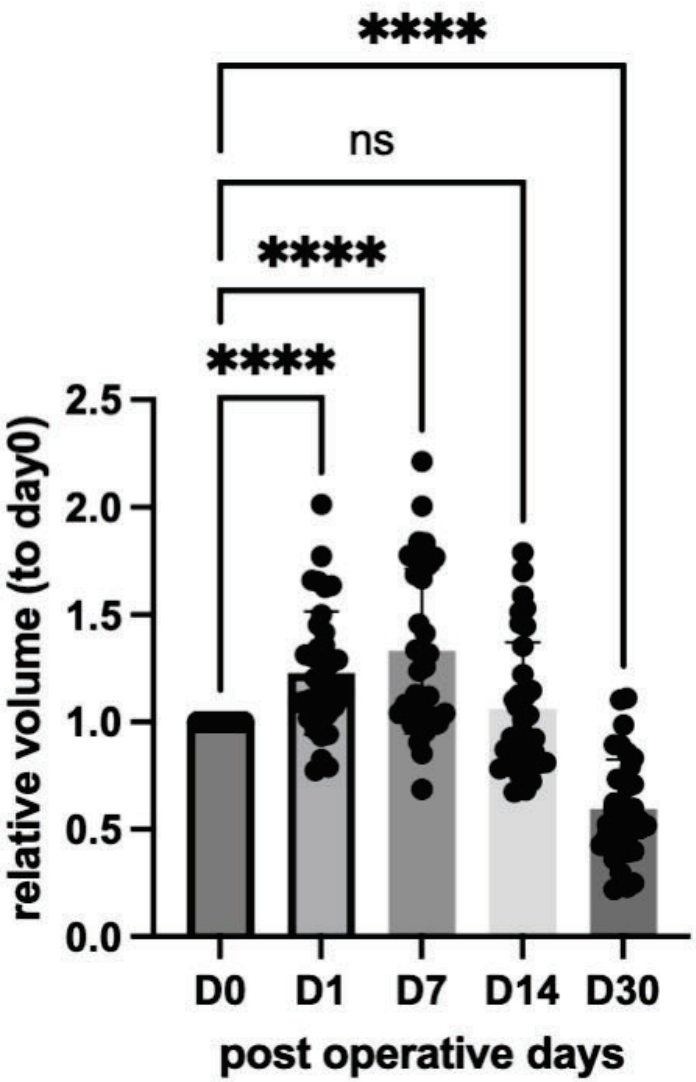

****
ns
****
****
relative volume (to day0)
2.5
2.0
1.5
1.0
0.5
0.0
D0
D1
D7
D14
D30
post operative days

Figure 2

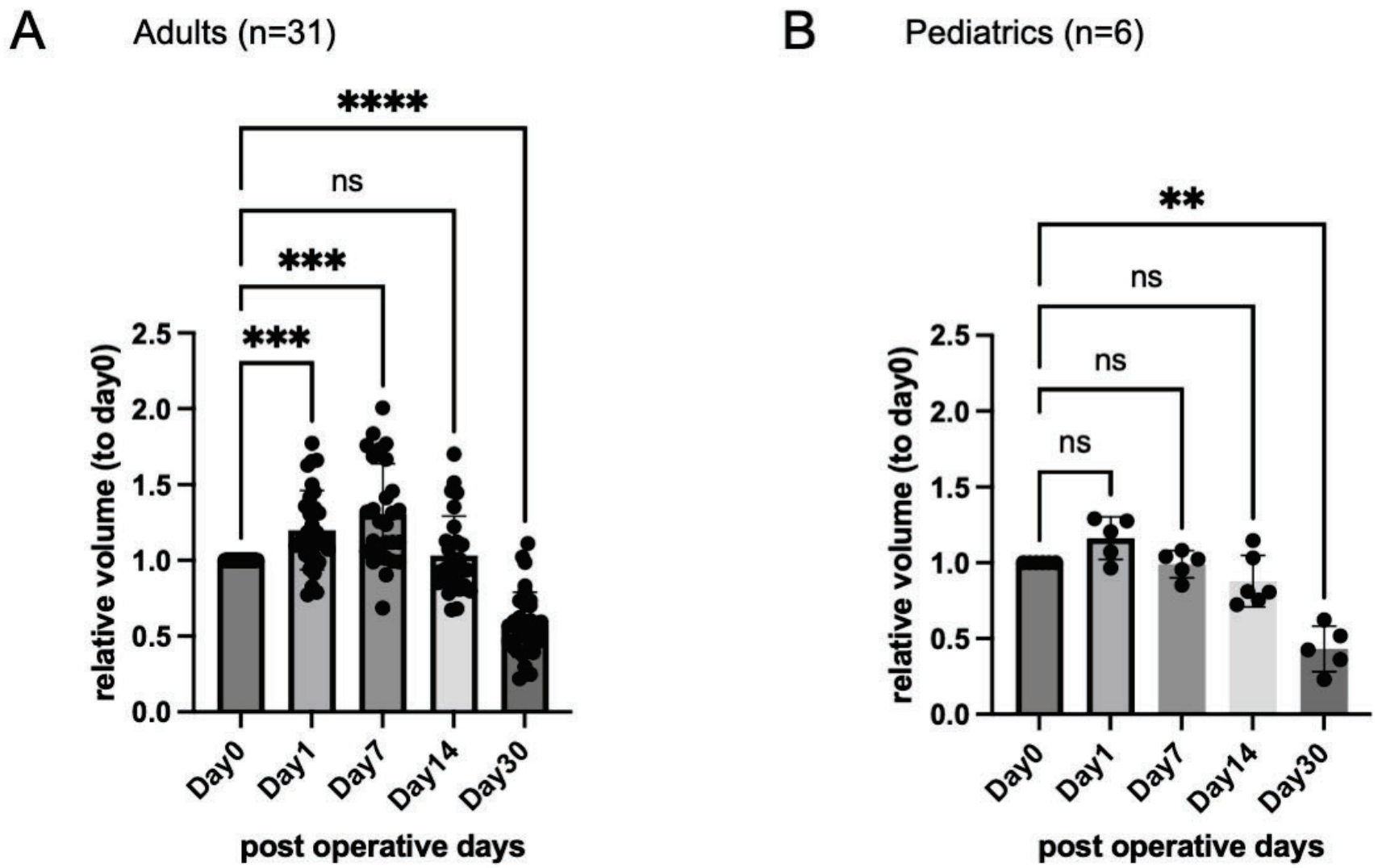
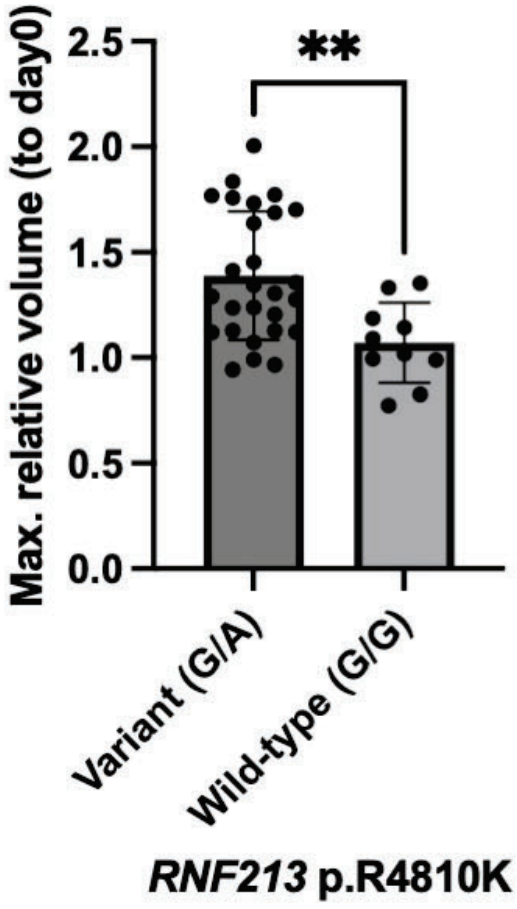

A
Adults (n=31)
****
ns
***
***
relative volume (to day0)
2.5
2.0
1.5
1.0
0.5
0.0
Day0
Day1
Day7
Day14
Day30
post operative days
B
Pediatrics (n=6)
**
ns
ns
ns
relative volume (to day0)
2.5
2.0
1.5
1.0
0.5
0.0
Day0
Day1
Day7
Day14
Day30
post operative days

Figure 3

A


**
Max. relative volume (to day0)
2.5
2.0
1.5
1.0
0.5
0.0
Variant (G/A)
Wild-type (G/G)
RNF213 p.R4810K

B

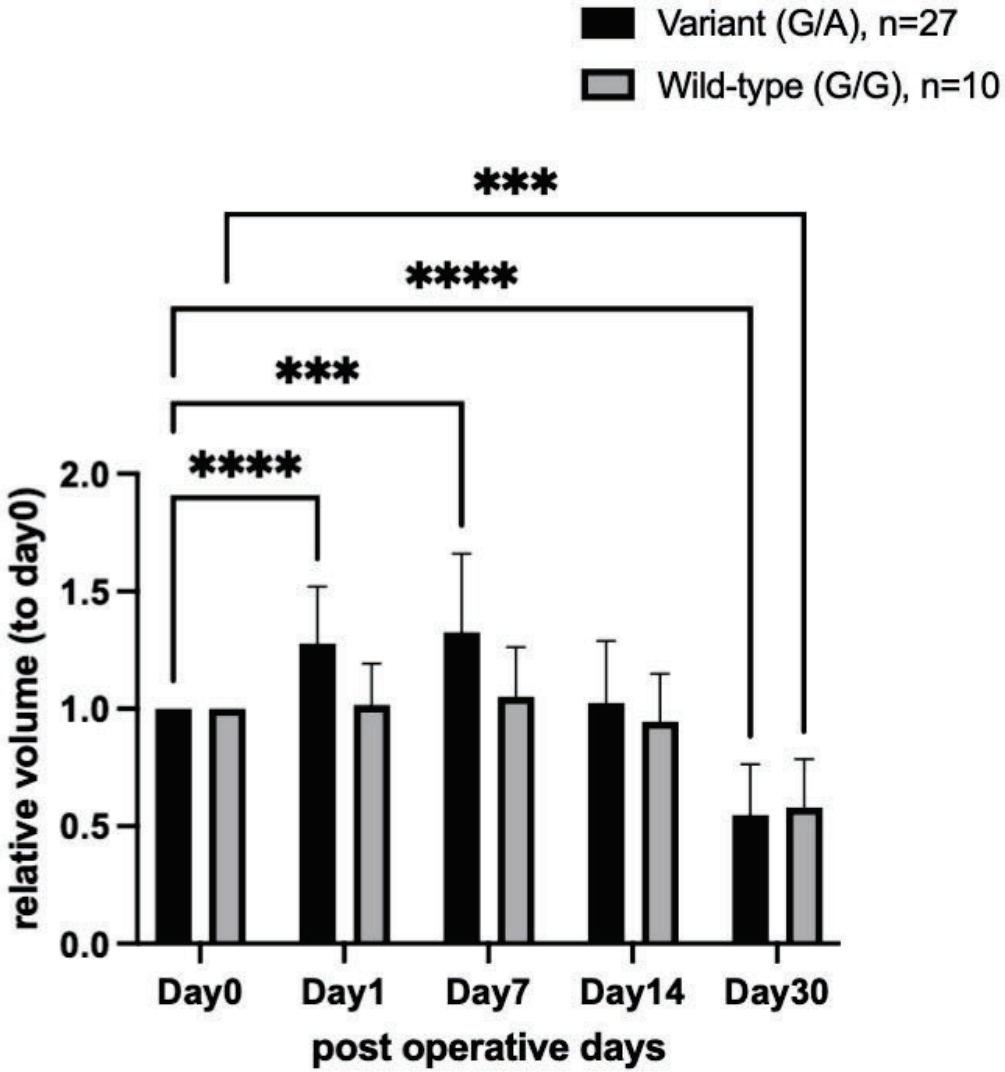
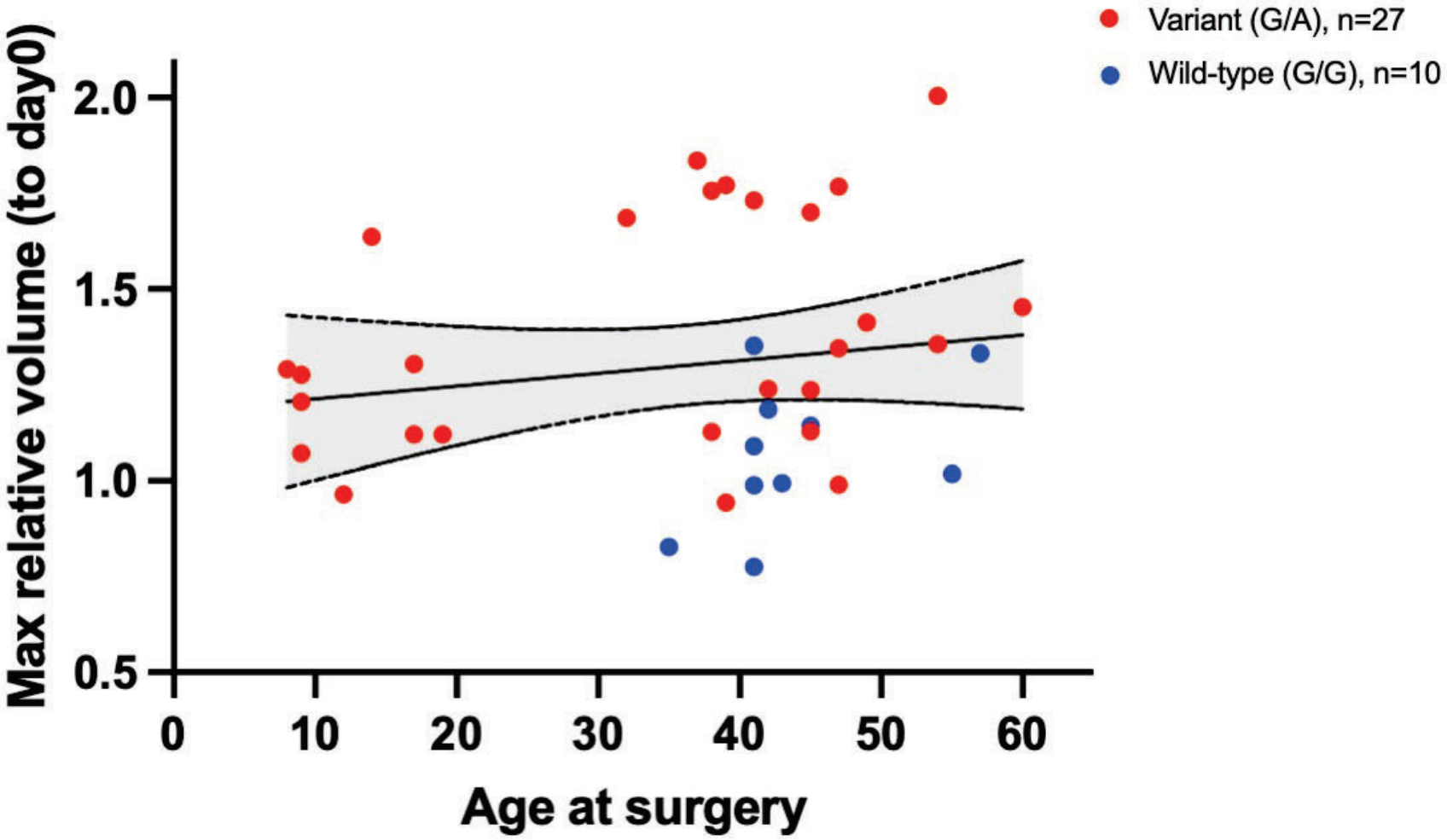

Variant (G/A), n=27
Wild-type (G/G), n=10
***
****
***
****
relative volume (to day0)
2.0
1.5
1.0
0.5
0.0
Day0
Day1
Day7
Day14
Day30
post operative days

Figure 4


Variant (G/A), n=27
Wild-type (G/G), n=10
Max relative volume (to day0)
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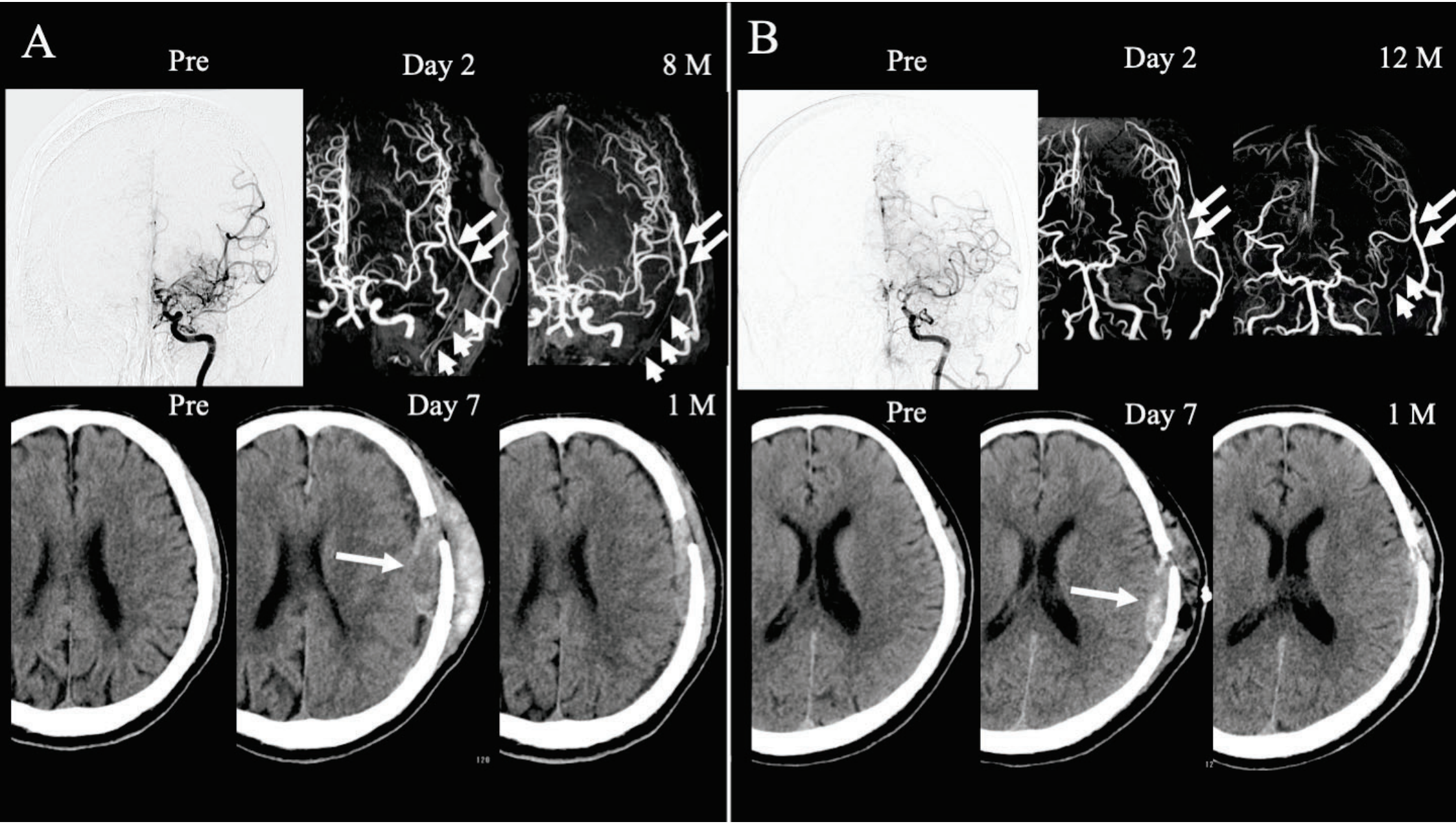
1.5
1.0
0.5
0
10
20
30
40
50
60
Age at surgery


A
Pre
Day 2
8 M
Pre
Day 7
1 M
B
Pre
Day 2
12 M
Pre
Day 7
1 M

Number of Hemispheres		37
Age at Surgery [years]		37±15
Age group		
	Adult (> or =16years)	31
	Pediatrics (< or =15years)	6
Male: Female		6:31
Right: Left		15:22
Clinical Presentation		
	TIA	19
	Infarct	14
	ICH	4
Suzuki's Angiographical Stage		
	3	23
	4	12
	5	0
	6	2
Comorbidities		
	none	22
	HT	13
	DM	3
	DLp	12
	pAf	1
Preoperative systemic immune inflammation index		668±546
RNF213 p.R4810K		
	G/A variant	27
	G/G wild-type	10
Preoperative antiplatelet agent		
	Aspirin	29
	Cilostazol	8
Combined bypass procedure		
	STA-MCA single anastomosis, EDMS	36
	STA-MCA double anastomosis, EDMS	1
Operation time [minutes]		245±35
Cranial window for muscle insertion [mm^2]		819±147

TABLE 1. Summary of baseline characteristics of all 37 hemispheres

TIA, transient ischemic attack; ICH, intracranial hemorrhage; HT, hypertension

DM, diabetes mellitus; DLp, dyslipidemia; pAf, paroxysmal atrial fibrillation

Values are expressed as the number of subjects or mean±standard deviation.

Variables	Maximal relative temporal muscle pedicle volume mean (95% confidence interval)	Univariate analysis P value	Multiple regression analysis Coefficient (β)	95% Confidence Interval	Significance
Age at surgery [years]		0.3372 β=0.003337	0.0037	-0.009221 to 0.01656	0.557
Age group		0.6051			
Adult (> or =16years)	1.31 (1.20-1.43)		NA		
Pediatrics (< or =15years)	1.24 (1.00-1.48)				
Sex		0.8691			
Male	1.32 (0.95-1.70)		0.31	-0.2238 to 0.8449	0.2379
Female	1.30 (1.19-1.41)				
Side		**0.0448**	0.124	-0.1393 to 0.3865	0.988
Left	1.39 (1.24-1.55)				
Right	1.19 (1.06-1.33)				
Clinical presentation (ICH)		0.4226			
ICH	1.18 (0.95-1.42)		-0.17	-0.5343 to 0.1986	0.3487
no ICH	1.32 (1.20-1.43)				
Clinical presentation (TIA)		**0.0319**	-0.0399	-0.3233 to 0.2436	0.776
TIA	1.20 (1.07-1.34)				
no TIA	1.42 (1.26-1.58)				
Clinical presentation (Infarct)		**0.0044**	0.224	-0.06396 to 0.5126	0.123
Infarct	1.49 (1.30-1.68)				
no Infarct	1.20 (1.09-1.31)				
Suzuki's angiographical stage		0.3062 β=-0.06407	0.0073	-0.1864 to 0.2009	0.9382
Comorbidities (HT)		0.6018			
HT	1.27 (1.07-1.47)		NA		
no HT	1.32 (1.19-1.45)				
Comorbidities (DM)		0.6198			
DM	1.22 (0.95-1.48)		-0.13	-0.5977 to 0.3468	0.5836
no DM	1.31 (1.20-1.42)				
Comorbidities (DLp)		0.5674			
DLp	1.26 (1.03-1.49)		NA		
no DLp	1.32 (1.21-1.44)				
Preoperative systemic immune inflammation index		0.749 β=0.000032	-1.20E-06	-0.0002447 to 0.0002423	0.9918
RNF213 p.R4810K		**0.0041**	**0.485**	**0.1449 to 0.8257**	**0.0078**
G/A variant	1.39 (1.27-1.51)				
G/G wild-type	1.07 (0.93-1.21)				
Preoperative antiplatelet agent		0.8734			
aspirin	1.30 (1.19-1.40)		0.071	-0.2775 to 0.4189	0.6746
cilostazol	1.32 (0.95-1.69)				
Operation time [minutes]		0.473 β=-0.001068	-0.0019	-0.005465 to 0.001653	0.2753
Cranial window for muscle insertion [mm2]		0.2549 β=0.0004063	-3.58E-05	-0.001156 to 0.001084	0.9472

TABLE 2. Analysis of factors associated with maximal relative temporal muscle pedicle volume after STAMCA anastmosis with EDMS in all the 37 hemiepshres
ICH, intracranial hemorrhage; TIA, traansient ischemic attack; HT, hypertension; DM, diabetes mellitus; DLp, Dyslipidemia; NA, not applicable

	Adults (n=31)	Pediatrics(n=6)	All(n=37)
Max. relative temporal muscle volume	1.3±0.33	1.2±0.23	1.3±0.31
Postoperative development of bypass			
direct bypass development	30 (97%)	6 (100%)	35 (95%)
indirect bypass development	21 (68%)	6 (100%)	27 (73%)
Recurrence of stroke			
TIA	0 (0%)	0 (0%)	0 (0%)
Infarct	0 (0%)	1 (17%)	1 (2.7%)
ICH	1 (3.2%)	0 (0%)	1 (2.7%)
Latest mRS			
0-2	30 (97%)	6 (100%)	36 (97%)
3-6	1 (3.2%)	0 (0%)	1 (2.7%)

TABLE 3. Surgical outcomes following combined bypass surgery

CBF = cerebral blood flow; ICH = intracranial hemorrhage; mRS = modified Rankin scale; TIA = transient ischemic attack

Values are expressed as the number of subjects or mean ± standard deviation.