Association of *RNF213* polymorphism and cortical hyperintensity sign on fluid-attenuated inversion recovery images after revascularization surgery for moyamoya disease: possible involvement of intrinsic vascular vulnerability

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Short Title: RNF213 and FLAIR cortical hyperintensity after revascularization for MMD

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Abstract

A cortical hyperintensity on fluid-attenuated inversion recovery images (FLAIR cortical hyperintensity, FCH) is an abnormal finding after revascularization surgery for moyamoya disease. This study aimed to investigate the pathophysiology of FCH through genetic analyses of *RNF213* p.R4810K polymorphism and perioperative hemodynamic studies using single photon emission computed tomography.

We studied 96 hemispheres in 65 adults and 47 hemispheres in 27 children, who underwent combined direct and indirect revascularization. Early or late FCH was defined when it was observed on postoperative days 0–2 or 6–9, respectively. FCH scores (range: 0–6) were evaluated according to the extent of FCH in the operated hemisphere.

FCHs were significantly more prevalent in adult patients than pediatric patients (early: 94% vs. 78%; late: 97% vs. 59%). In pediatric patients, FCH scores were significantly improved from the early to late phase regardless of the *RNF213* genotype (mutant median [IQR]: 2 [1 - 5] vs. 1 [0 - 2]; wild-type median: 4 [0.5 - 6] vs. 0.5 [0 - 1.75]). In adults, FCH scores were significantly improved in patients with the wild-type *RNF213* allele (median: 4 [2 - 5.25] vs. 2 [2 - 3]); however, they showed no significant improvement in patients with the *RNF213* mutation. FCH scores were significantly higher in patients with symptomatic cerebral hyperperfusion than those without it (early median: 5 [4 - 5] vs. 4 [2 - 5]; late median: 4 [3 - 5] vs. 3 [2 - 4]).

In conclusion, the *RNF213* p.R4810K polymorphism was associated with prolonged FCH, and extensive FCH was associated with symptomatic cerebral hyperperfusion in adult patients with moyamoya disease.

Introduction

Moyamoya disease (MMD) is a steno-occlusive cerebrovascular disorder, characterized by progressive occlusion of the supraclinoid internal carotid artery that results in the formation of an abnormal vascular network [1]. Although the cause of the disease is not completely elucidated, a susceptibility gene ring finger protein 213 (*RNF213*) has been identified in East Asian populations [2, 3]. Additionally, a recent study indicates *RNF213* as a key regulator of cerebral endothelium integrity [4]. In MMD, direct revascularization surgery, such as superficial temporal artery–middle cerebral artery (STA–MCA) anastomosis, is a widely performed standard treatment that reduces the risk of future ischemic and hemorrhagic strokes [5-7]. Although direct revascularization surgery effectively improves cerebral hemodynamics immediately after surgery, cerebral hyperperfusion (CHP) is a potential complication in the early postoperative period, and it can cause transient neurological deficits or intracerebral hemorrhage [8-10]. *RNF213* p.R4810K (rs112735431, c.14576G>A) polymorphism was reported to be a predictor of prolonged/delayed CHP after direct revascularization surgery [11]. Furthermore, a correlation between the polymorphism and good development of indirect surgical collaterals has been reported [12, 13]. Thus, vascular vulnerability, enhanced vascular permeability, and abnormal angiogenesis associated with the *RNF213* p.R4810K polymorphism are hypothesized to be involved in these acute and chronic postoperative conditions [11, 14-16].

In MMD, an abnormal hyperintensity sign is observed on fluid-attenuated inversion recovery (FLAIR) in the cortex of the operated hemisphere (FLAIR cortical hyperintensity, FCH) after direct revascularization surgery. Although an association between extensive FCH and transient neurological deficits has been reported [17, 18], the underlying mechanisms of FCH are not fully understood, and the effect of the *RNF213* genotype on FCH has not been investigated. Therefore, the present study aimed to identify the pathophysiology of FCH, through *RNF213* genetic analyses and perioperative hemodynamic studies, in pediatric and adult patients with MMD.

Methods

Patients and surgical procedures

This study included consecutive patients who underwent combined direct and indirect revascularization for MMD at our hospital and submitted written informed consent to genetic analysis of *RNF213* p.R4810K polymorphism between 2006 and 2020.

Surgical revascularization was considered for patients with hemodynamic compromise or for patients with hemorrhagic presentation. The surgical procedure has been described previously [19]. Direct revascularization procedures, including STA–MCA anastomosis, as well as indirect bypass procedures, such as encephalo-duro-arterio-myo-synangiosis, were performed in all hemispheres.

Genetic analysis of the *RNF213* p.R4810K polymorphism

Peripheral blood samples were obtained from the patients, and the Taqman single-nucleotide polymorphism genotyping assay (Applied Biosystems; Foster City, CA, USA) was performed to determine the *RNF213* p.R4810K allelic type, as described previously [12, 20].

FCH

Magnetic resonance (MR) studies, including diffusion-weighted imaging (DWI), FLAIR, T2* weighted imaging (T2*WI), and MR angiography (MRA), were routinely performed preoperatively and at postoperative days 0–2 and 6–9 using a clinical 3.0-T scanner. These were done to evaluate the perioperative conditions. FCH was defined as intraparenchymal hyperintensity in the cortex of a surgically treated hemisphere in FLAIR images. Absence of acute infarction or hemorrhage in the area was confirmed using DWI or T2*WI. Early and late FCH were defined when they were observed at postoperative day 0–2 and 6–9, respectively. The ivy sign was differentiated and excluded in this study. The ivy sign represents FLAIR high intensity in cortical vessels and indicates slow blood flow, and is often separated from the cortex or crosses the cortex. (**Figure 1A and Supplementary figure 1**) [21].

The extent of FCH was reviewed using all slices of axial FLAIR images. The FCH score was defined by modifying a previously described method [17]. The frontal lobe was assigned to the anterior, and the parietal and temporal lobes were assigned to the posterior. For each part, the extent of the FCH was scored as 0 (not visible), 1 (limited to one-third of the part), 2 (extending from one-third to two-thirds of the part), or 3 (extending over two-thirds of the part). The total FCH score was the sum of the anterior and posterior scores (minimum = 0, maximum = 6) (**Figure 2B and Supplementary figure 2**). The FCH scores were determined through the agreement of two authors (HU and MI), who were blinded to the genetic analyses of each case.

Perioperative management and cerebral blood flow (CBF) examinations

Presurgical regional CBF was quantitatively measured using ^{123}I N-isopropyl-p-iodoamphetamine single-photon emission computed tomography (^{123}I-IMP SPECT). Postoperative CBF measurements were performed on postoperative days 0–2 and 7. Postoperative CHP was defined as a focal and intense increase in CBF, followed by its normalization in subsequent ^{123}I-IMP SPECT exams [10]. The evaluation of the ^{123}I-IMP SPECT was performed through visual assessments by two authors (HU and KK). Both authors reached a consensus on their evaluations. Postsurgical systolic blood pressure was maintained below 140 mmHg with strict blood pressure monitoring and control using intravenous antihypertensive drugs, if necessary. When a transient neurological deficit was observed, MRI and MRA were performed to check the patency of the direct bypass and for any fresh lesions. When ^{123}I-IMP SPECT showed CHP without new lesions in the area that corresponded to the neurological deficit, it was considered as symptomatic CHP.

Data analyses

The unpaired-*t* test and Mann–Whitney test were used, respectively, to compare continuous and ranked variables between two groups. Categorical variables were compared using the χ^2 test. The level of significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism (GraphPad Software; San Diego, CA, USA).

Results

Demographic data

The subjects were 96 hemispheres in 65 adults (≥18 years old at the time of surgery) and 47 hemispheres in 27 children. The clinical data including mean age, sex, surgical side, initial presentation, and *RNF213* genotype are shown in **Table 1**. There were no significant differences in terms of these factors between the *RNF213*-mutant and -wild-type groups in both pediatric and adult patients (data not shown).

Incidence of postoperative FCH and *RNF213* genotypes

FCH was not observed preoperatively (within one month) in any case. Early FCH occurred in 79% and 75% of *RNF213*-mutant and -wild-type pediatric patients and 93% and 97% of *RNF213*-mutant and -wild-type adult patients, respectively. Late FCH occurred in 62% and 50% of *RNF213*-mutant and -wild-type pediatric patients and in 98% and 94% of *RNF213*-mutant and -wild-type adult patients, respectively. Thus, the incidences of early and late FCH were not significantly associated with the *RNF213* genotype in both pediatric and adult patients. However, overall incidences of early and late FCH were significantly higher in adult patients than in pediatric patients (early: 94% vs. 78%, $p < 0.01$; late: 97% vs. 59%, $p < 0.001$). (**Supplementary figure 2**).

Temporal change in postoperative FCH for each *RNF213* genotype

Among the pediatric patients, FCH scores were significantly improved from early to late phase in both the *RNF213*-mutant (median [interquartile ratio, IQR]: 2 [1 - 5] vs. 1 [0 - 2], $p < 0.01$) and -wild-type (median [IQR]: 4 [0.5 - 6] vs. 0.5 [0 - 1.75], $p < 0.01$) groups (**Figure 2A, left**). In adults, FCH scores were significantly improved from early to late phase in patients without *RNF213* mutation (wild-type patients) (median [IQR]: 4 [2 - 5.25] vs. 2 [2 -3], $p < 0.001$); however, FCH scores showed no significant improvement from early to late phase in patients carrying an *RNF213* mutation (**Figure 2A, right**).

Association between postoperative CHP and FCH

Symptomatic and asymptomatic CHP occurred in 11% and 19% of pediatric patients and 24% and 43% of adult patients, respectively (**Table 1**). The incidences of symptomatic and asymptomatic

CHP were not significantly different between the wild-type and mutant *RNF213* allele-carrying pediatric and adult patients (Symptomatic: 8% (3/36) vs 17% (2/12), $p = 0.59$; Asymptomatic: 22% (8/36) vs 8% (1/12), $p = 0.42$). Late FCH scores were significantly higher in adult patients with radiological (symptomatic and asymptomatic) CHP or symptomatic CHP than those without these conditions (median [IQR]: 4 [3 -5] vs. 3 [2 - 4], $p < 0.05$; median [IQR]: 4 [3 - 5] vs. 3 [2 - 4], $p < 0.01$**, Figure 2B**, respectively). On the other hand, FCH scores were not associated with the occurrence of radiological CHP or symptomatic CHP in pediatric patients (median [IQR]: 1 [0 - 2] vs. 1 [0 - 2], $p > 0.05$; median [IQR]: 2 [0 - 3.5] vs. 1 [0 - 2], $p > 0.05$, respectively).

Discussion

This is the first study to identify an association between *RNF213* genotypes and FCH after revascularization surgery for MMD. Our findings showed that mutation in *RNF213* was significantly correlated with prolonged FCH in adult patients. The present study also demonstrated that FCH is observed quite frequently in both pediatric and adult patients undergoing combined revascularization surgery. The high incidence of FCH strongly suggests an intrinsic pathological background for MMD.

The mechanisms underlying the development of FCH after revascularization surgery for MMD are unclear, but we speculate that vasogenic edema is dominantly involved in this intrinsic phenomenon. The absence of DWI-high intensity in the corresponding area of FCH supports this hypothesis [22, 23]. Takemoto et al. demonstrated that the postoperative cerebral blood volume increase was correlated with the occurrence of FCH; they considered that the underlying mechanism of FCH was vasogenic edema, which is associated with impairment of the blood–brain barrier (BBB) and leakage of fluid into the brain parenchyma [18]. Previous studies have shown that patients with MMD intrinsically have vascular vulnerability associated with BBB impairment. First, serum and plasma cytokine analysis revealed that patients with MMD showed significantly higher expression of vascular endothelial growth factor and matrix metalloproteinase 9, which have potential roles in increasing the permeability of the BBB, than healthy subjects [15, 24]. Second, histological analysis of surgically collected MCA specimens from MMD patients showed significantly thinner media than control specimens, implying anatomical fragility in the intracranial arteries [25]. Third, intraoperative videoangiography using sodium fluorescein extravasation demonstrated that MMD patients had BBB impairment [14]. These findings suggest that the intrinsic vascular vulnerability in MMD may contribute to the formation of vasogenic edema or FCH after revascularization surgery.

Although the molecular functions of *RNF213* and its effect on postoperative vasogenic edema need to be elucidated, *RNF213* is known to play a vital role in endothelial cells and vascular smooth muscle cells, contributing to the functional maintenance of these vascular cells through controlling inflammation cascades [20, 26]. Therefore, mutation in *RNF213* can make these vascular cells

vulnerable to secondary insults. In fact, Tashiro et al. demonstrated a correlation between *RNF213* mutations and prolonged/delayed CHP after combined direct and indirect revascularization surgery for MMD, strongly suggesting that *RNF213* mutations affect vascular integrity in the postoperative pathophysiology [11]. Similarly, in the present study, prolonged FCH in adult patients with *RNF213* mutations also suggests an additional *RNF213*-related vascular vulnerability to postoperative hemodynamic changes.

Furthermore, the present study identified that the occurrence of CHP is significantly associated with extensive FCH in adult patients. In contrast, an association between CHP and FCH was not observed in pediatric patients. Several studies have analyzed the relationship between FCH and postoperative CBF increase. Takemoto et al. and Hamano et al. reported that a postoperative CBF increase was not related to the extension of FCH [17, 18]. One reason for this discrepancy would be that pediatric and adult patients were analyzed together in these studies; the patterns of postoperative cerebral hemodynamic changes and the frequency of CHP are quite different between pediatric and adult patients [10]. Therefore, the present study evaluated pediatric and adult patients separately, which suggested involvement of some age-related vascular factors in adult patients because pediatric patients showed improvement of FCH regardless of the *RNF213* genotype. Further study is warranted to elucidate the precise mechanism of the difference in postoperative cerebral hemodynamics between pediatric and adult patients.

The present study has several limitations. First, the FCH scores were determined by the agreement of two neurosurgeons, who were blinded to genetic analyses and clinical outcomes, and accurate inter-observer variability was not evaluated; variability could have been obtained if the judgments had been performed independently. Second, MR images analyzed in the current study were acquired by several MR scanners. Difference in machine venders and imaging parameters might slightly affect the image quality and the FCH scores. Third, apparent diffusion coefficients (ADC) in the area of FCH were not quantitatively evaluated, because it was difficult to precisely set region of interest and evaluate quantitative ADCs in the linear limited area. Further quantitative analyses are warranted in future to confirm the mechanism of FCH. However, ADCs typically seem to be high in the area of FCH when compared with the preoperative hemispheres or those without FCH (Supplementary figure 1). This indicates the mechanism of FCH is predominantly vasogenic edema rather than cytogenic edema. Forth, the present study did not indicate which postoperative managements should be conducted against FCH or whether there were any long-term effects of FCH on patients' clinical outcomes, such as cognitive function. However, postoperative managements against CHP, such as precise hemodynamic examinations and adequate control of blood pressure, are important [27] because the present study suggests that CHP and FCH share common pathophysiology.

Conclusions

In this study, FCH was frequently observed after combined direct and indirect revascularization surgery in pediatric and adult patients with MMD, regardless of the *RNF213* genotype. In adult patients, prolonged FCH and extensive FCH were significantly associated with the *RNF213* p.R4810K polymorphism and CHP, respectively. These findings suggest that intrinsic and *RNF213*-related vascular vulnerabilities to postoperative hemodynamic change are involved in the pathogenesis of FCH.

Statement of Ethics

The present study conforms to the guidelines issued in the Declaration of Helsinki. This study was approved by the Institutional Ethics Committees (approval number 14–053).

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Conflicts of Interest

All authors declare that there is no personal or institutional financial interest in drugs, materials, or devices described in this article.

Author Contributions

Uchino and Fujimura: conception and study design. Uchino, Tokairin, Tatezawa, Sugiyama, and Ito: acquisition of data. Uchino, Ito, and Kazumata: analysis and interpretation. Uchino: drafting. Uchino, and Fujimura: critical revision of the article. Fujimura: study supervision.

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Figure legends

Figure 1. (A) Representative images of ivy sign (upper) and FLAIR cortical hyperintensity (lower). T2*WI shows absence of subarachnoid hemorrhage and DWI shows absence of hyperintensity respectively in the area of FCH in the same case. (B) Radiological findings of a 34-year-old woman who underwent right-sided combined revascularization for MMD. Genetic analysis indicates the presence of *RNF213* p.R4810K polymorphism. (Left) Preoperative FLAIR images show no FCH, and SPECT scans demonstrate decrease in CBF in the bilateral hemispheres. (Middle) FLAIR images on postoperative day 1 show FCH in the right hemisphere; the FCH score is 3 (anterior 1, posterior 2). (Right) On postoperative day 7, FCH is detected in a wider area than on day 1, when the FCH score was 5 (anterior 3, posterior 2). SPECT scans demonstrate focal increase in CBF in the right hemisphere while she had no neurological deficits postoperatively. Dot circles show the extent of FCH.

Figure 2. Box plots illustrating early and late FCH scores in each group of pediatric and adult patients (A), and late FCH scores in each group of adult patients with and without symptomatic CHP (B). Gray and white boxes show patients with and without the *RNF213* p.R4810K polymorphism (mutant and wild-type), respectively. The boxes indicate the median and interquartile range (IQR). The bars and dots indicate 1.5 IQRs and outliers, respectively. $^{**}p < 0.01$, $^{****}p < 0.0001$

Supplementary figure 1. Images of a representative case of the right-sided revascularization. FLAIR and ADC images of preoperative, postoperative day 2 and day 7. While FCH is limited on day 2, it is prominent in the frontal lobe on day 7. ADCs seem to be high in the area of FCH (arrows).

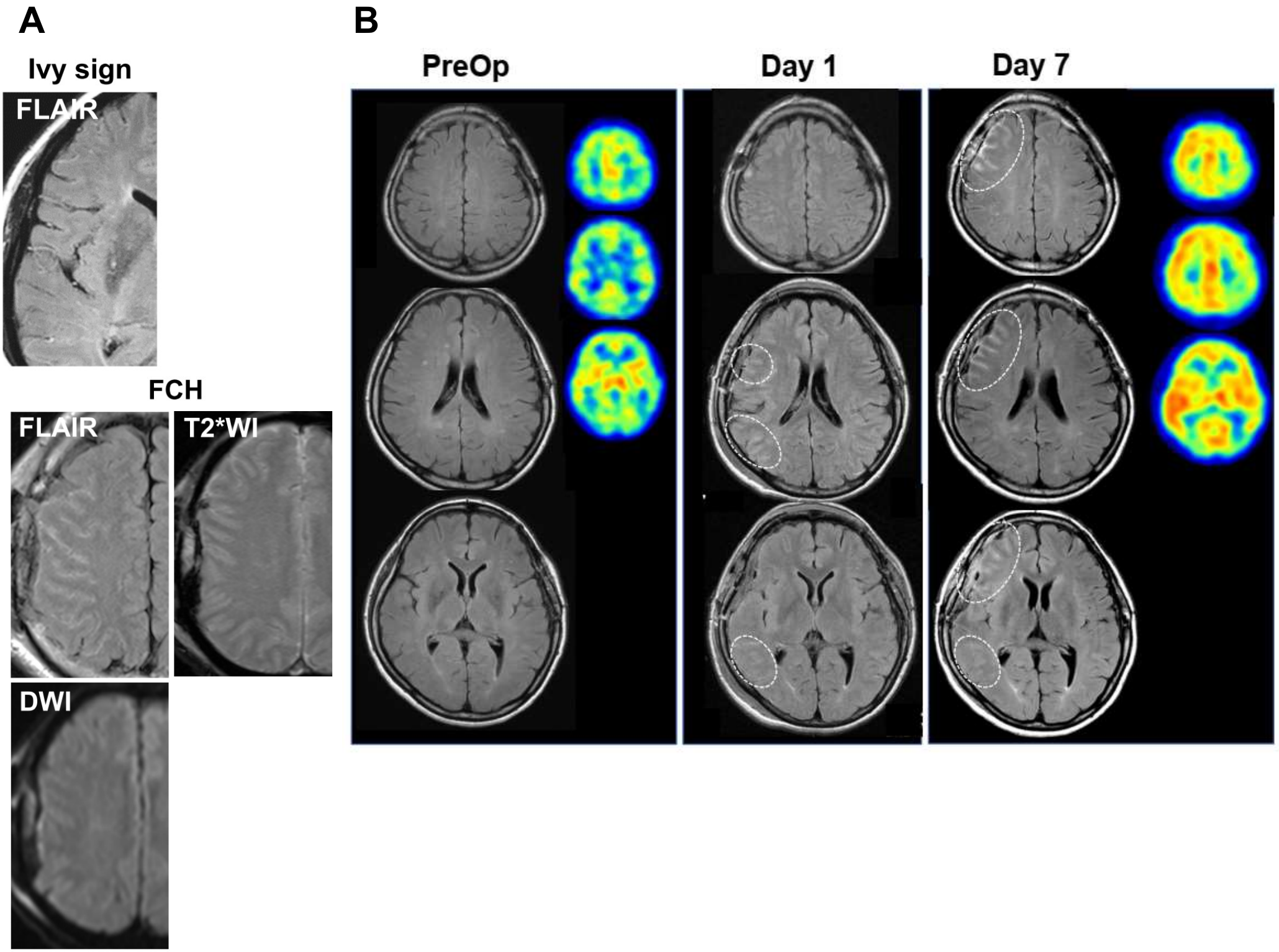
Supplementary figure 2. Examples of the FCH score.

Supplementary figure 3. Bar graphs showing the incidences of early and late FCH in pediatric and adult patients. Gray and white columns show hemispheres with and without the *RNF213* p.R4810K polymorphism (mutant and wild-type), respectively. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$

Table 1. Summary of clinical data in patients included in this study

	Pediatric patients	Adult patients
Hemispheres, n	47	96
Mean age (range), y	8.4 (1-15)	42.7 (19-67)
Male/female	24/23	23/73
Rt/Lt-sided surgery, n	25/22	49/47
Clinical type, n		
Ischemia	43	74
Hemorrhage	2	16
Others	2	6
RNF213 genotype		
AA/AG/GG, n	1 (2%)/34 (72%)/12 (26%)	0 (0%)/62 (65%)/34 (35%)
Symptomatic CHP, n	5 (11%)	23 (24%)
Asymptomatic CHP, n	9 (19%)	41 (43%)

CHP; cerebral hyperperfusion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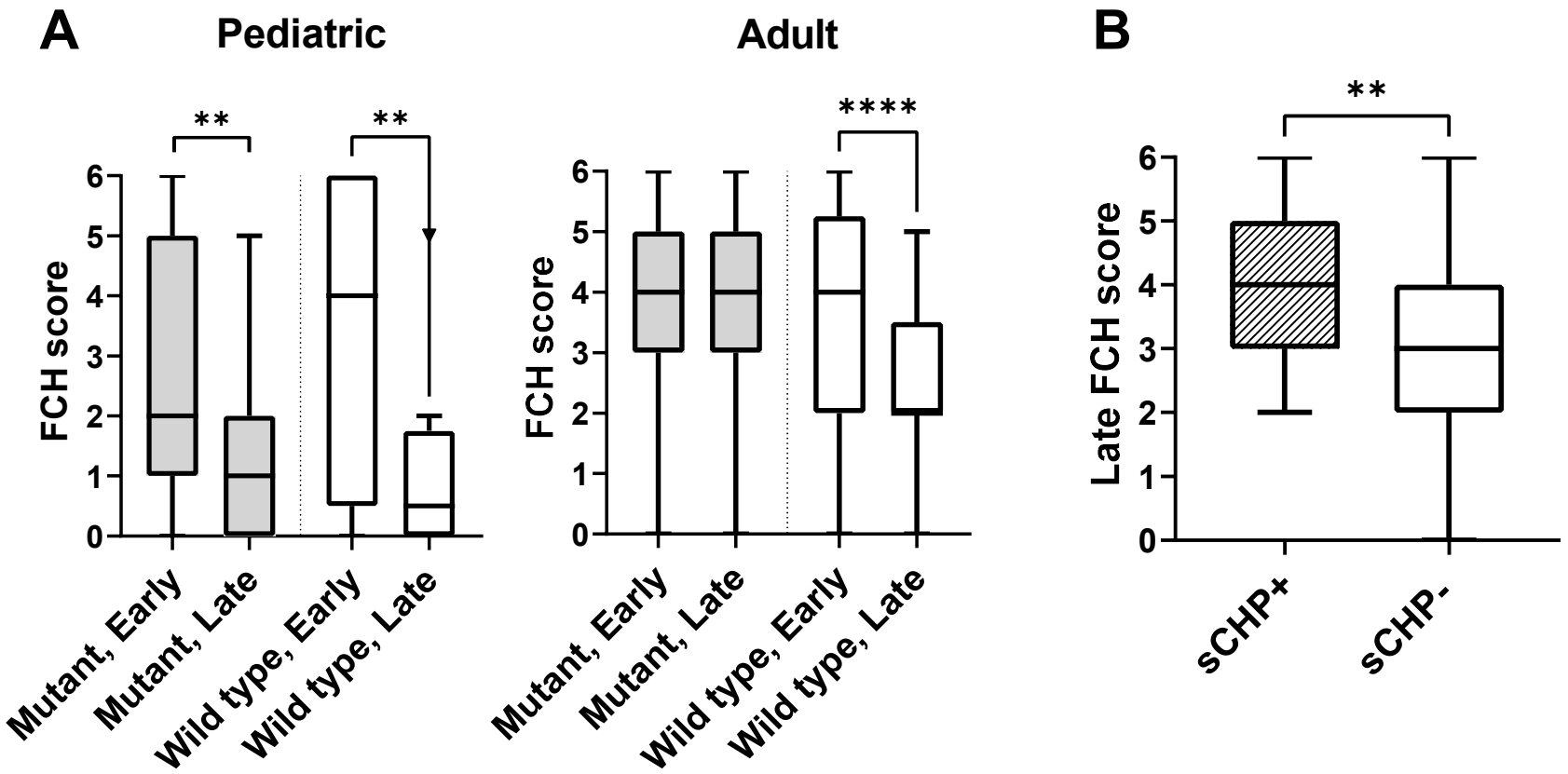
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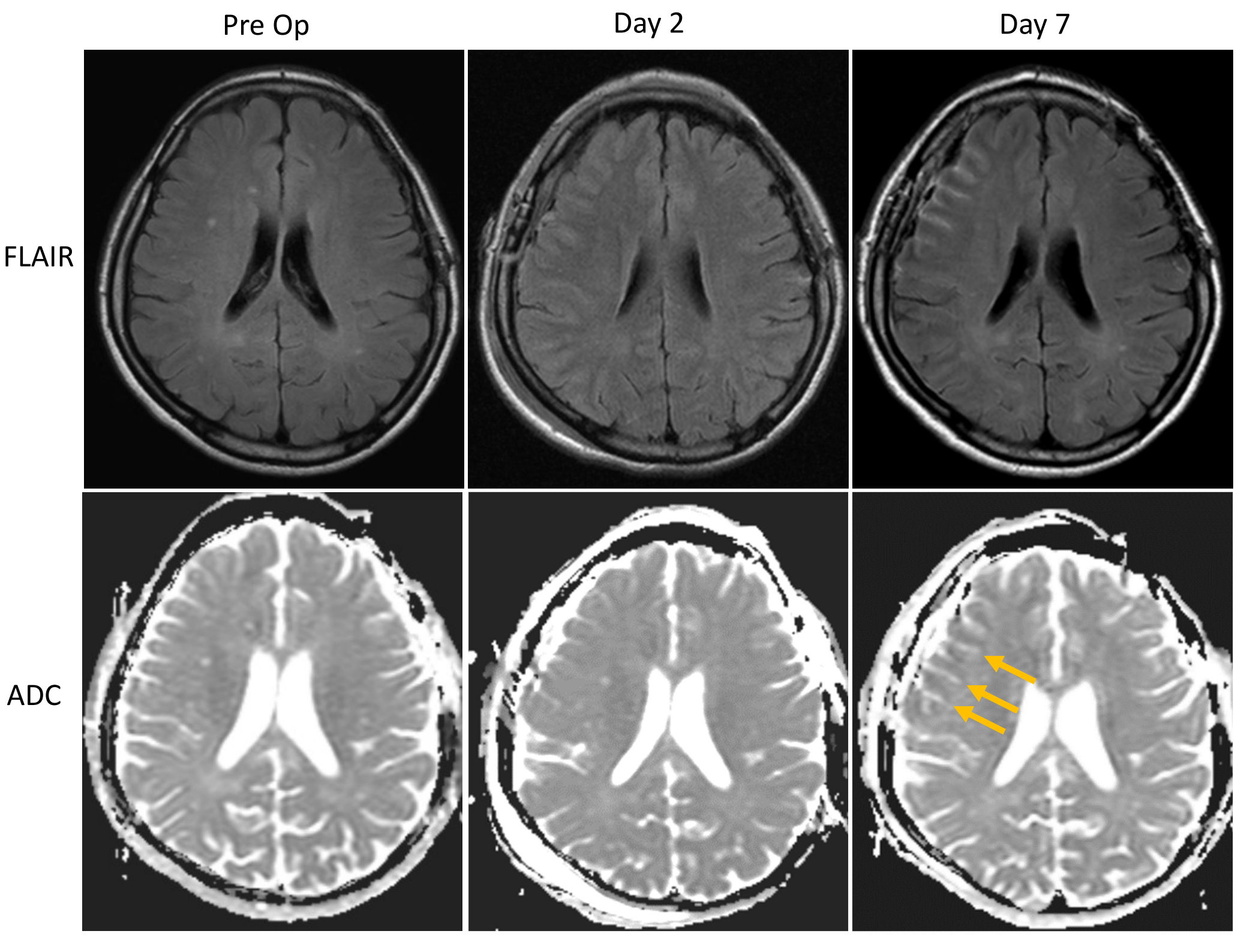
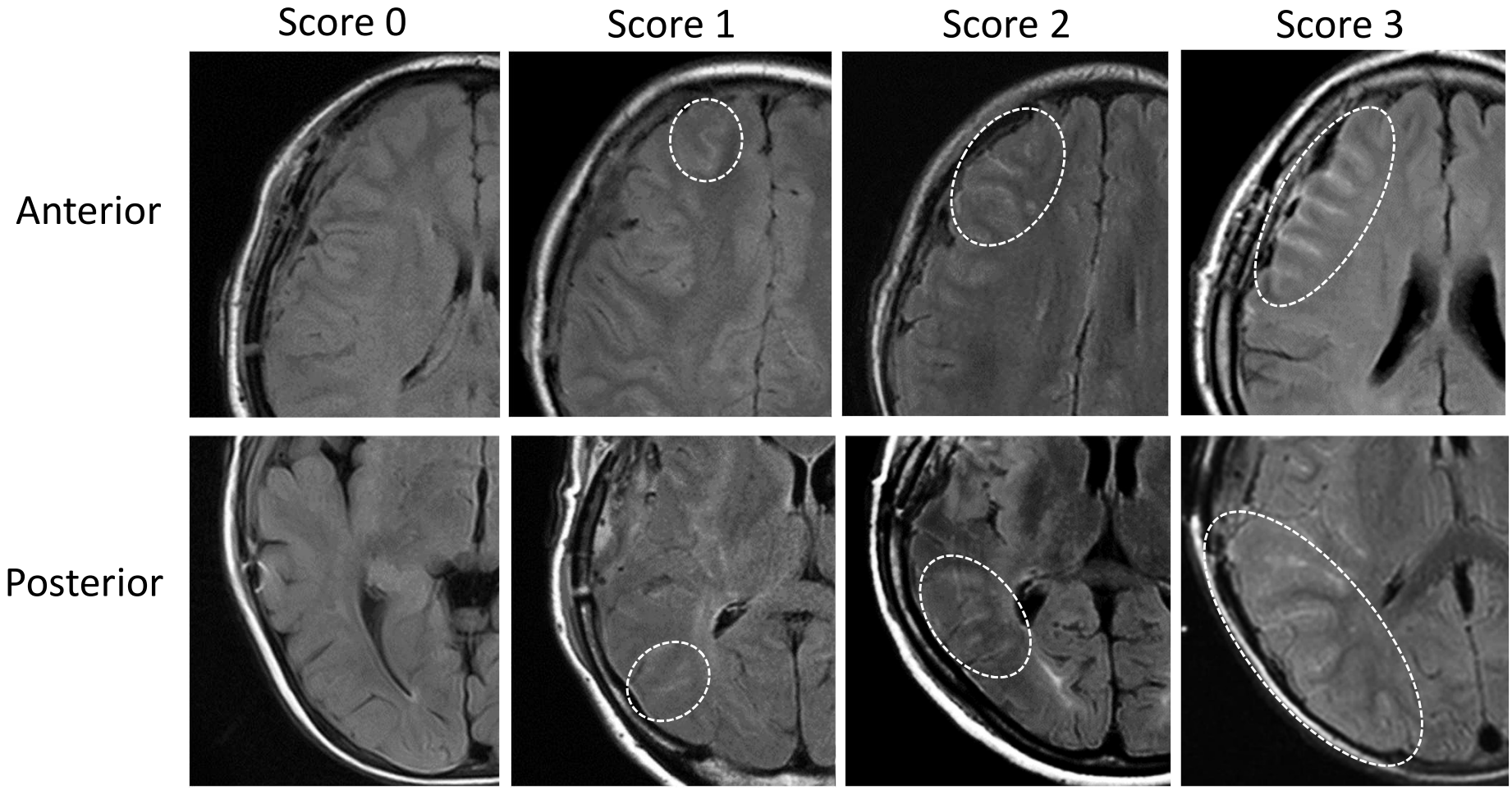
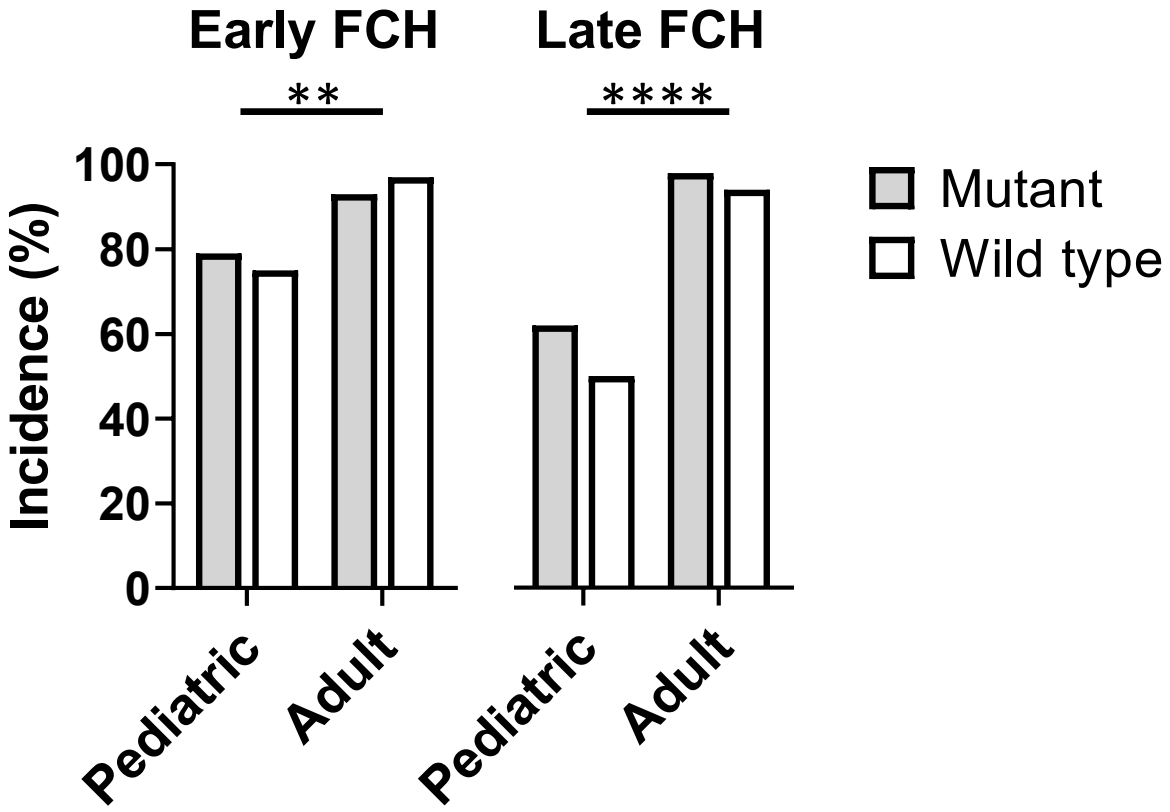

Pre Op
Day 2
Day 7
FLAIR
ADC


Score 0
Score 1
Score 2
Score 3
Anterior
Posterior


Early FCH
Late FCH
**
****
Incidence (%)
100
80
60
40
20
0
Pediatric
Adult
Pediatric
Adult
Mutant
Wild type