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

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9
10 Short Title: RNF213 and FLAIR cortical hyperintensity after revascularization for MMD

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24

25 **Abstract**

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

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

35 FCHs were significantly more prevalent in adult patients than pediatric patients (early: 94% vs.
36 78%; late: 97% vs. 59%). In pediatric patients, FCH scores were significantly improved from the
37 early to late phase regardless of the *RNF213* genotype (mutant median [IQR]: 2 [1 - 5] vs. 1 [0 - 2];
38 wild-type median: 4 [0.5 - 6] vs. 0.5 [0 - 1.75]). In adults, FCH scores were significantly improved
39 in patients with the wild-type *RNF213* allele (median: 4 [2 - 5.25] vs. 2 [2 - 3]); however, they
40 showed no significant improvement in patients with the *RNF213* mutation. FCH scores were
41 significantly higher in patients with symptomatic cerebral hyperperfusion than those without it
42 (early median: 5 [4 - 5] vs. 4 [2 - 5]; late median: 4 [3 - 5] vs. 3 [2 - 4]).

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

46

47

48 **Introduction**

49 Moyamoya disease (MMD) is a steno-occlusive cerebrovascular disorder, characterized by
50 progressive occlusion of the supraclinoid internal carotid artery that results in the formation of an
51 abnormal vascular network [1]. Although the cause of the disease is not completely elucidated, a
52 susceptibility gene ring finger protein 213 (*RNF213*) has been identified in East Asian populations
53 [2, 3]. Additionally, a recent study indicates *RNF213* as a key regulator of cerebral endothelium
54 integrity [4]. In MMD, direct revascularization surgery, such as superficial temporal artery–middle
55 cerebral artery (STA–MCA) anastomosis, is a widely performed standard treatment that reduces the
56 risk of future ischemic and hemorrhagic strokes [5-7]. Although direct revascularization surgery
57 effectively improves cerebral hemodynamics immediately after surgery, cerebral hyperperfusion
58 (CHP) is a potential complication in the early postoperative period, and it can cause transient
59 neurological deficits or intracerebral hemorrhage [8-10]. *RNF213* p.R4810K (rs112735431,
60 c.14576G>A) polymorphism was reported to be a predictor of prolonged/delayed CHP after direct
61 revascularization surgery [11]. Furthermore, a correlation between the polymorphism and good
62 development of indirect surgical collaterals has been reported [12, 13]. Thus, vascular vulnerability,
63 enhanced vascular permeability, and abnormal angiogenesis associated with the *RNF213* p.R4810K
64 polymorphism are hypothesized to be involved in these acute and chronic postoperative conditions
65 [11, 14-16].

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

73

74 **Methods**

75 **Patients and surgical procedures**

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

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

83

84 **Genetic analysis of the *RNF213* p.R4810K polymorphism**

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

88

89 **FCH**

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

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

108

109 **Perioperative management and cerebral blood flow (CBF) examinations**

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

121

122 **Data analyses**

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

128 **Results**

129 **Demographic data**

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

136 **Incidence of postoperative FCH and *RNF213* genotypes**

137 FCH was not observed preoperatively (within one month) in any case. Early FCH occurred in 79%
138 and 75% of *RNF213*-mutant and -wild-type pediatric patients and 93% and 97% of *RNF213*-mutant
139 and -wild-type adult patients, respectively. Late FCH occurred in 62% and 50% of *RNF213*-mutant
140 and -wild-type pediatric patients and in 98% and 94% of *RNF213*-mutant and -wild-type adult
141 patients, respectively. Thus, the incidences of early and late FCH were not significantly associated
142 with the *RNF213* genotype in both pediatric and adult patients. However, overall incidences of early
143 and late FCH were significantly higher in adult patients than in pediatric patients (early: 94% vs.
144 78%, $p < 0.01$; late: 97% vs. 59%, $p < 0.001$). (**Supplementary figure 2**).

146 **Temporal change in postoperative FCH for each *RNF213* genotype**

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

155 **Association between postoperative CHP and FCH**

156 Symptomatic and asymptomatic CHP occurred in 11% and 19% of pediatric patients and 24% and
157 43% of adult patients, respectively (**Table 1**). The incidences of symptomatic and asymptomatic

158 CHP were not significantly different between the wild-type and mutant *RNF213* allele-carrying
159 pediatric and adult patients (Symptomatic: 8% (3/36) vs 17% (2/12), $p = 0.59$; Asymptomatic: 22%
160 (8/36) vs 8% (1/12), $p = 0.42$). Late FCH scores were significantly higher in adult patients with
161 radiological (symptomatic and asymptomatic) CHP or symptomatic CHP than those without these
162 conditions (median [IQR]: 4 [3 - 5] vs. 3 [2 - 4], $p < 0.05$; median [IQR]: 4 [3 - 5] vs. 3 [2 - 4], $p <$
163 0.01 , **Figure 2B**, respectively). On the other hand, FCH scores were not associated with the
164 occurrence of radiological CHP or symptomatic CHP in pediatric patients (median [IQR]: 1 [0 - 2]
165 vs. 1 [0 - 2], $p > 0.05$; median [IQR]: 2 [0 - 3.5] vs. 1 [0 - 2], $p > 0.05$, respectively).
166

167 **Discussion**

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

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

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

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

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

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

230

231 **Conclusions**

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

238

239 **Statement of Ethics**

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

242

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247

248 **Conflicts of Interest**

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

251

252 **Author Contributions**

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

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340 symptomatic cerebral hyperperfusion after direct revascularization surgery for moyamoya disease,
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342

343 **Figure legends**

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

355

356 **Figure 2.** Box plots illustrating early and late FCH scores in each group of pediatric and adult
357 patients (A), and late FCH scores in each group of adult patients with and without symptomatic
358 CHP (B). Gray and white boxes show patients with and without the *RNF213* p.R4810K
359 polymorphism (mutant and wild-type), respectively. The boxes indicate the median and interquartile
360 range (IQR). The bars and dots indicate 1.5 IQRs and outliers, respectively. ** $p < 0.01$, **** $p <$
361 0.0001

362

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

367

368 **Supplementary figure 2.** Examples of the FCH score.

369

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

373

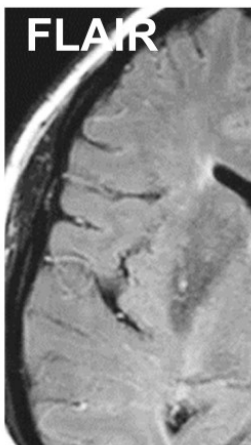
Table 1. Summary of clinical data in patients included in this study

	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
<i>RNF213</i> 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%)

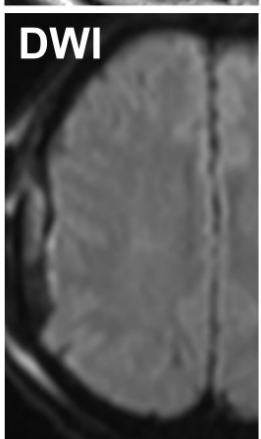
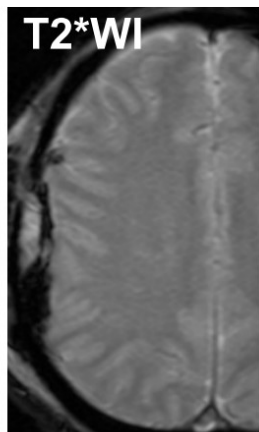
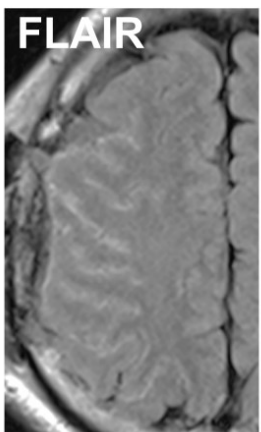
CHP; cerebral hyperperfusion

A

Ivy sign



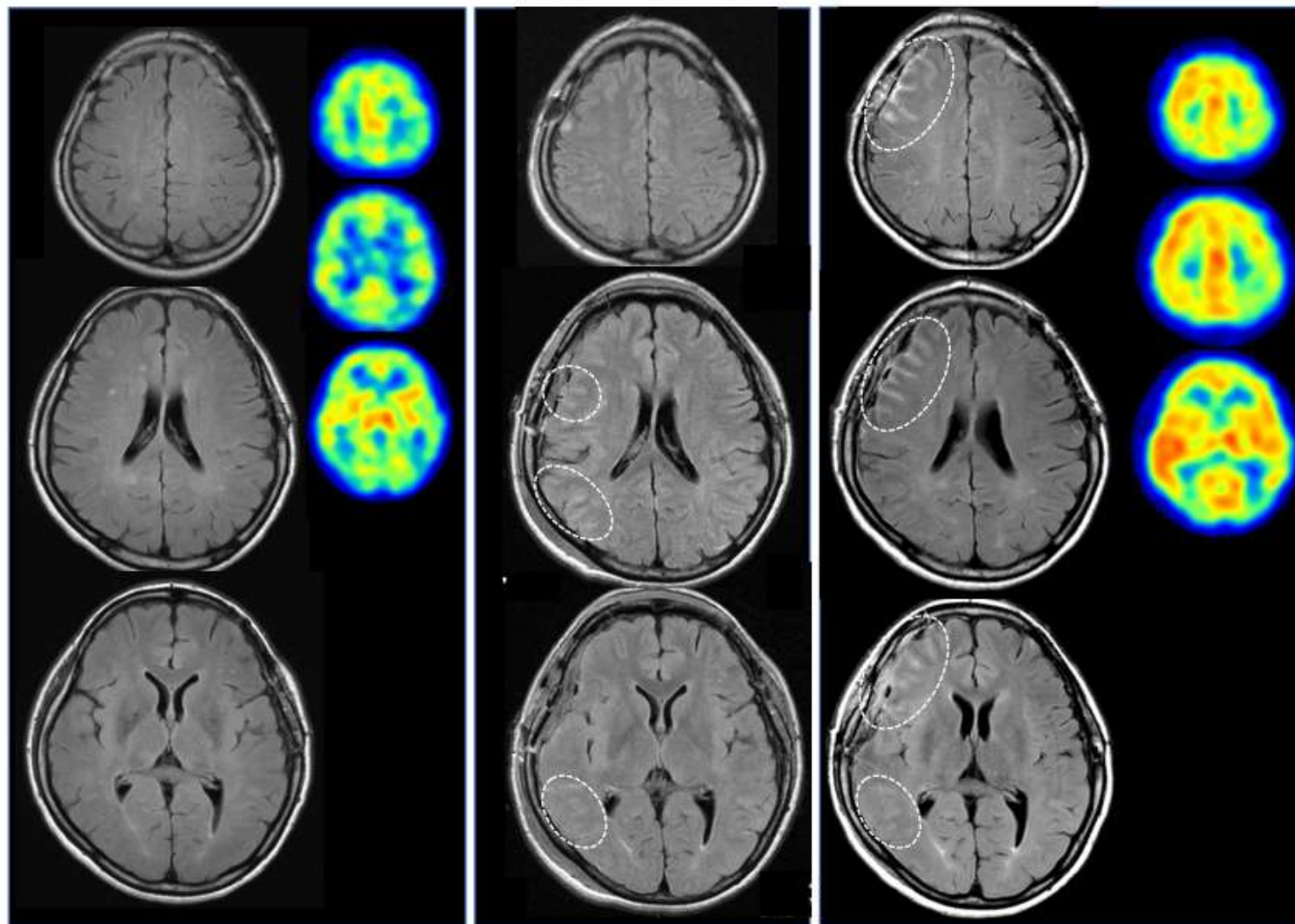
FCH

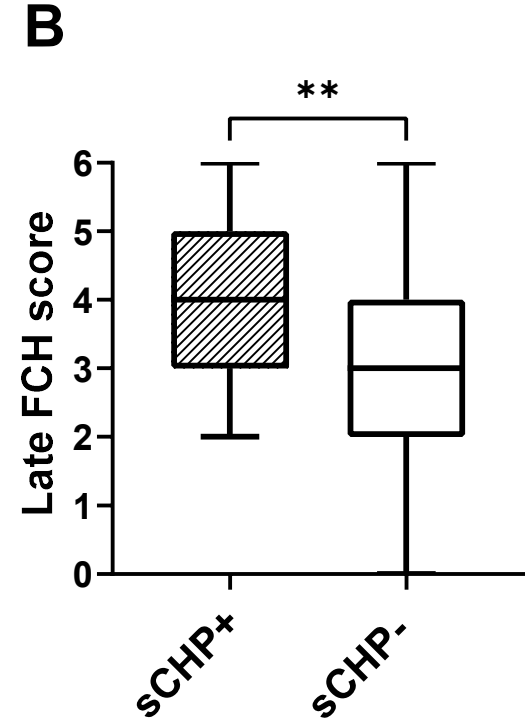
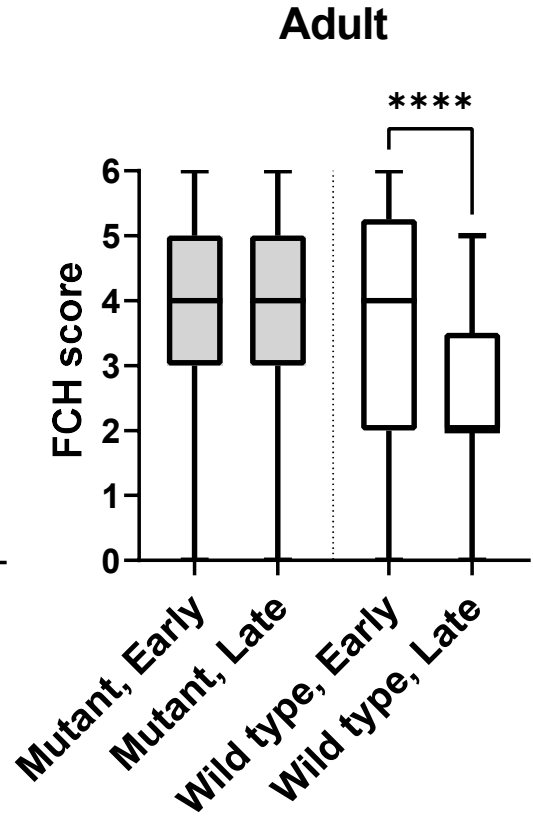
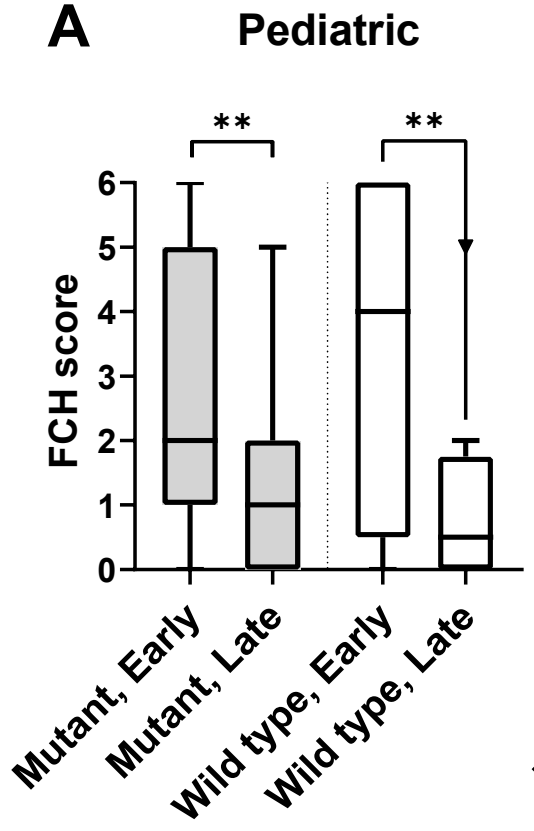
**B**

PreOp

Day 1

Day 7



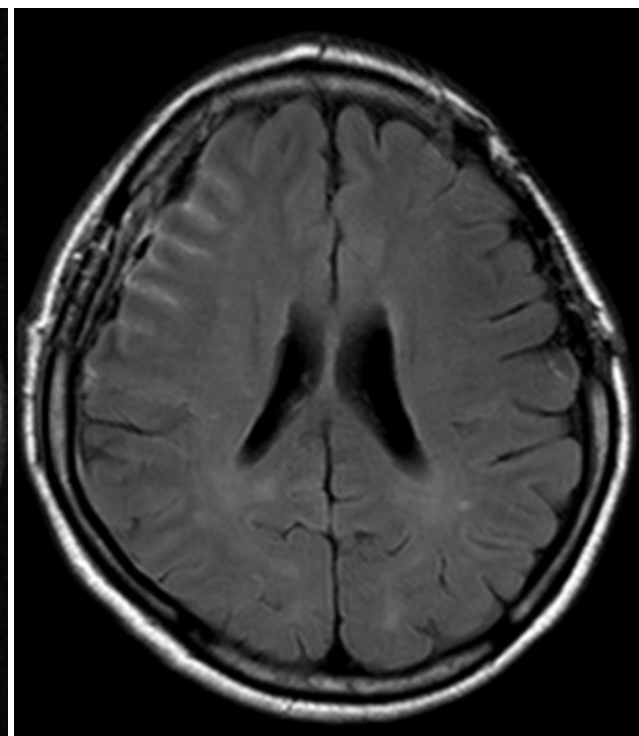
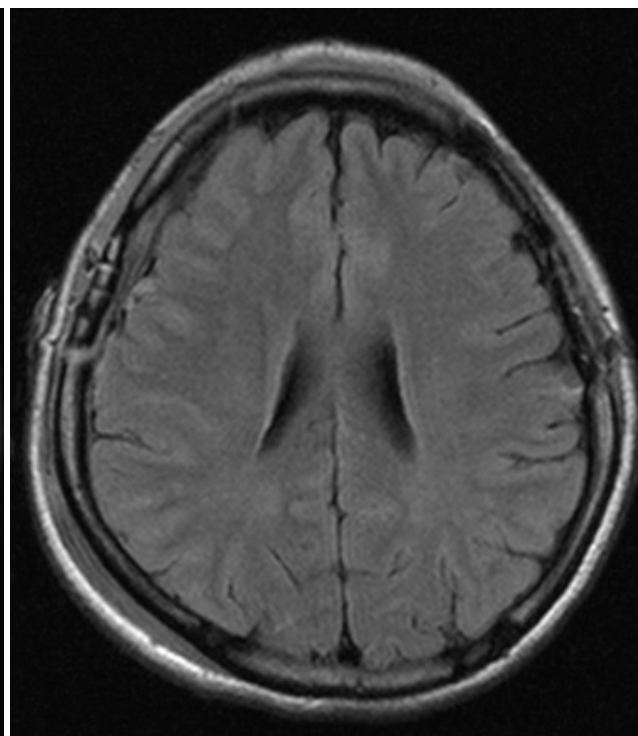
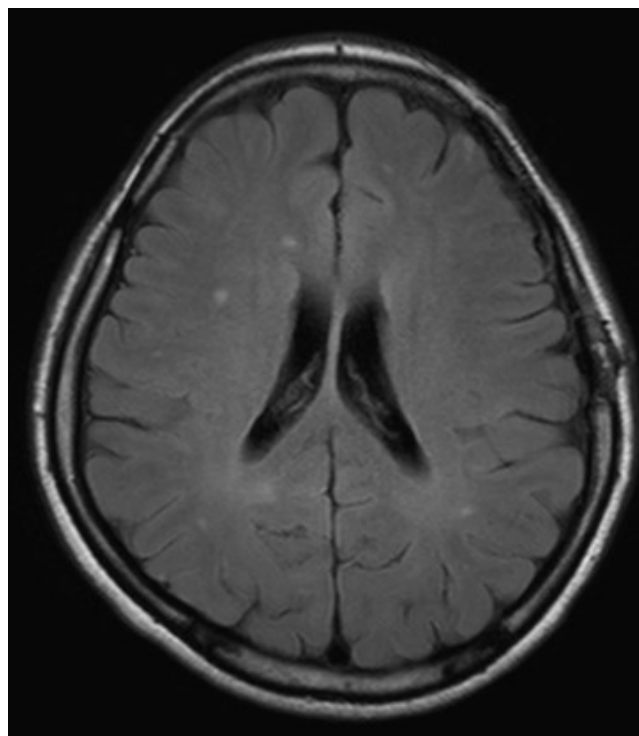


Pre Op

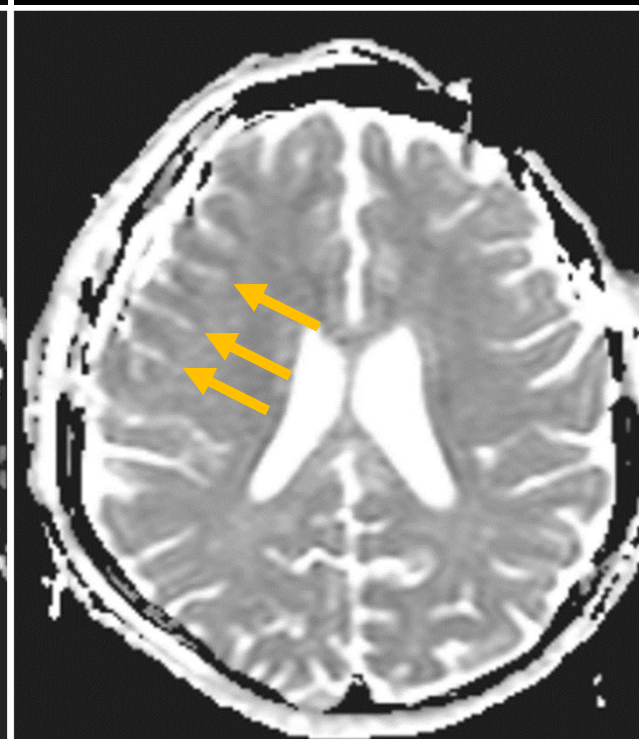
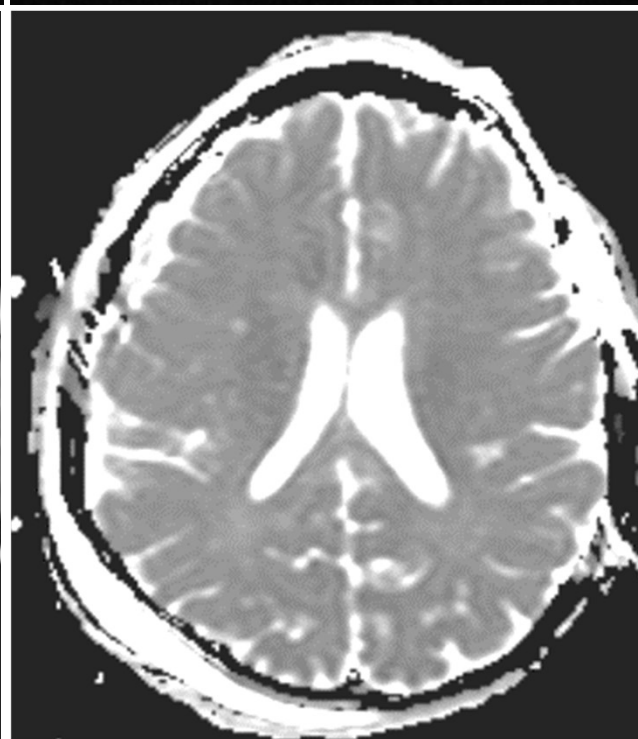
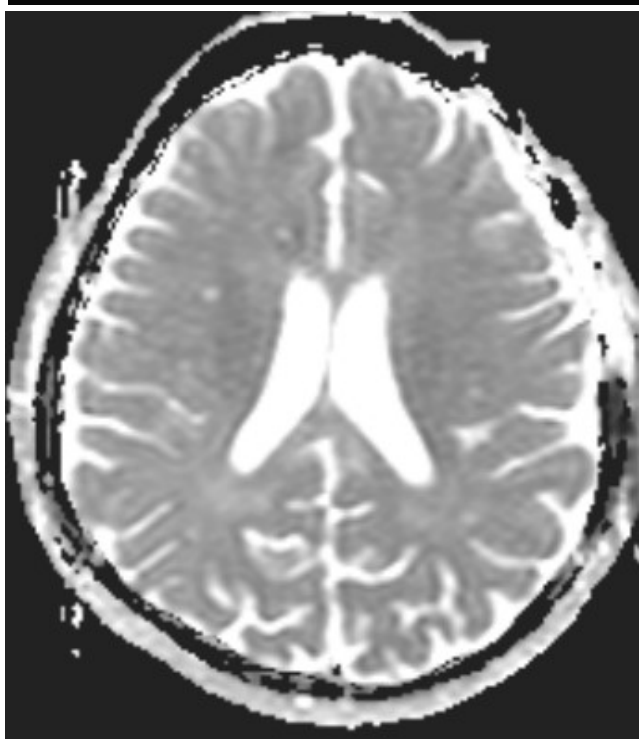
Day 2

Day 7

FLAIR



ADC



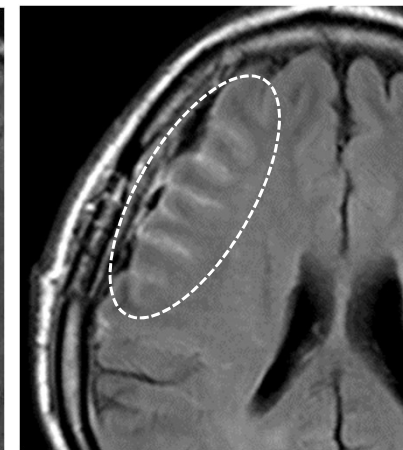
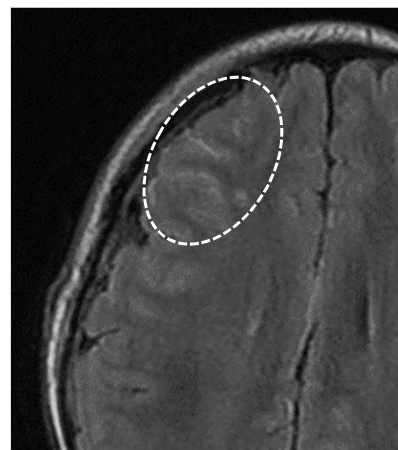
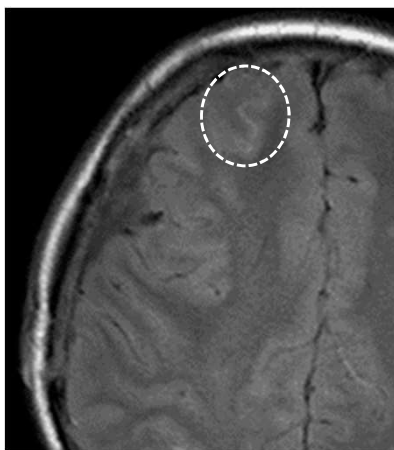
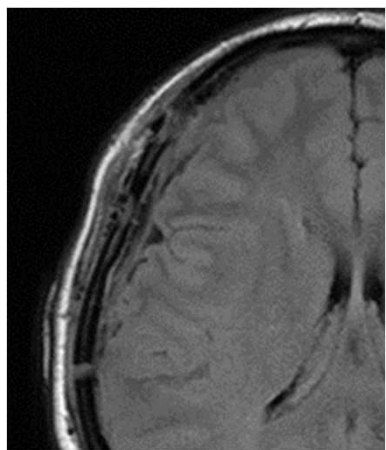
Score 0

Score 1

Score 2

Score 3

Anterior



Posterior

