Activating *KRAS* Mutations in Arteriovenous Malformations of the Brain: Frequency and Clinicopathologic Correlation

David S. Priemer MD[1], Alexander O. Vortmeyer MD[1], Shaobo Zhang MD[1], Hsim Yee Chang MLS(ASCP)[1], Kendra L. Curless MLS(ASCP)[1], Liang Cheng MD[1]

[1]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, 46202.

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Address correspondence and reprint requests to Liang Cheng, MD, Director of Molecular Pathology Laboratory, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 350 West 11th Street, IUHPL Room 4010, Indianapolis, IN 46202, USA. Telephone: 317-491-6442; Fax: 317-491-6419; E-mail: liang_cheng@yahoo.com

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ABSTRACT

Arteriovenous malformations (AVM) of the brain are considered congenital. Most AVMs are presumably sporadic, however rare familial cases occur and they may be observed in certain genetic disorders. We sought to determine the frequency of *KRAS* mutations and their association with clinicopathologic characteristics. We searched our neuropathology database from 2014-2017 for resected AVMs of the brain or dura mater. Twenty-one AVMs were tested (12 females, 9 males; average age: 32 years). *KRAS* mutations were found in 6/21 cases (28.5%). Five mutations were p.G12V, and one p.G12C. The *KRAS*-mutant group contained 4 females and 2 males, with an average age of 28 years, compared to 34 years in the non-mutant group (p=0.54). The average AVM size in the *KRAS*-mutant group was 3.9 cm, compared to 3.1 cm in the non-mutant group (p=0.52). There were no histologic differences between *KRAS*-mutant and non-mutant cases. In summary, *KRAS* mutations occur in almost one third of brain AVMs. *KRAS* p.G12V was the most common mutation identified. We also demonstrate the first reported instance of a *KRAS* p.G12C mutation in a brain AVM. The mean age of patients with *KRAS*-mutant AVMs was lower than the non-mutant group, and the mean size larger. Histologic characteristics were equally distributed between *KRAS*-mutant and non-mutant groups.

Highlights

- Most brain arteriovenous malformations are presumably sporadic, but rare familial cases have been reported.

- *KRAS* mutations occur in almost one third of brain arteriovenous malformations (AVMs).

- All observed clinical and histologic data of brain AVMs were equally distributed and similarly present in all AVMs, regardless of *KRAS* status.

INTRODUCTION

Brain arteriovenous malformations (AVM) are fistulous aggregations of arterial and venous vessels, are considered to be congenital, and may present across a large age range [1]. They have an incidence of up to approximately 2 per 100,000 person-years [2], and are responsible for approximately 4% of intracerebral hemorrhages, including up to one-third of intracerebral hemorrhages in young adults [3]. Most brain AVMs are presumably sporadic, but rare familial cases have been reported [4] and they may be observed in genetic disorders such as hereditary hemorrhagic telangiectasia [5], capillary malformation-arteriovenous malformation syndrome [6], and Wyburn-Mason syndrome [7].

Recently, Nikolaev et al. reported *KRAS* mutations in tissue from 45/72 brain AVMs (62.5%), but none within 21 paired blood samples [8]. In the same study, *KRAS* mutations were detected in endothelial cells from human brain AVMs in vitro and it was noted that mutant *KRAS* expression initiated increased ERK activity that was counteracted by inhibition of mitogen-activated protein kinase (MAPK)-ERK signaling. In addition, based on immunohistochemistry of 25 AVMs, Nikolaev et al. concluded that activation of the MAPK-ERK pathway is a defining molecular signature of AVMS. The initiating events for AVMs without RAS mutations are uncertain, however the pathogenetic mechanisms resulting from RAS-initiated activation of the MAPK-ERK pathway are mediated by angiogenic genes (e.g. *VEGF A/C*) and proteins in the notch signaling pathway.[9-12] In this study, we postulated that these mechanisms may result in potentially distinguishable clinical and/or histologic features between *KRAS*-mutant and non-mutant AVMs.

METHODS

Cases

A search of our neuropathology archives for intracranial AVMs was performed from 2014-2017. Hematoxylin and eosin (H&E) -stained and Verhoeff-van Gieson-stained slides were reviewed to confirm the diagnosis according to published texts.[13, 14] Slides were also examined to tissue adequacy and viability for molecular testing. Cases were excluded from molecular testing if the tissue amount was insufficient or if the tissue was felt to be extensively damaged by artifact (e.g. cautery).

Reviews of electronic clinical records were performed for patient age, gender, presenting symptoms, and past medical history (with particular reference to history of additional intracranial or extracranial vascular lesions). Imaging studies were reviewed to document the size and location of the AVM nidus. Family histories were also reviewed, particularly for history of brain AVMs, other vascular lesions, or stroke.

After identification of *KRAS*-mutant and non-mutant cases, H&E-stained and Verhoeff-van Gieson-stained slides were reexamined for differing histology between groups. Immunohistochemical stains for Ki-67 (Clone MIB-1; Dako, Santa Clara, CA) were also performed. The principle features considered in this examination included general diagnostic architectural characteristics, evidence of proliferative activity (mitoses, Ki-67 labelling in endothelial cells and vascular smooth muscle cells), and proportion of vascular channels with and without elastic laminae (either partial or complete circumferential staining). This research was approved by the Institutional Review Board.

KRAS Mutational Analysis

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were retrieved from each included case. *KRAS* mutation analysis was performed in a Clinical Laboratory Improvements and Amendments (CLIA) certified molecular pathology laboratory. DNA extraction from each block was performed using the Qiagen QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). DNA concentration was determined using the NanoDrop Spectrophotometer and adjusted to approximately 10 ng/µl in ddH2O. Polymerase chain reaction (PCR) testing was performed according to the recommended procedure using the Qiagen therascreen *KRAS* RGQ PCR Kit on the Qiagen Rotor-Gene Q MDx instrument. The therascreen *KRAS* RGQ PCR Kit provides eight separate PCR amplification reactions: seven mutation-specific reactions in codons 12 and 13 of exon 2 of the *KRAS* oncogene, and a wild-type control in exon 4. Analysis of crossing thresholds and mutation calls for each PCR amplification reaction were performed by the Rotor-Gene Q therascreen *KRAS* Assay Package once runs were completed.

Statistical Analysis

Student's t-tests were performed to assess for significance between patient age, gender, and AVM sizes in *KRAS*-mutant and non-mutant groups. Fisher's exact test was used to compare categorical data for clinicopathologic characteristics between *KRAS* mutated and non-mutant groups. Statistical significance was defined as $p < 0.05$ and all p values were two-tailed.

RESULTS

Twenty-one AVM resections diagnosed by our neuropathology service from 2014-2017 were determined to be viable and sufficient for molecular testing. Slide review confirmed the AVM diagnosis in all cases. This 21-case cohort included material from 12 females and 9 males.

The average patient age was 32 years (median: 32 years; range: 9-65 years). **Table 1** contains a summary of data from each case.

Clinical records revealed that only one patient, an 11 year-old girl with a left frontal lobe AVM, had a history of an additional vascular malformation that was within the parotid gland. No patients had multiple intracranial AVMs. No patients had a family history of intracranial AVMs or extracranial vascular lesions. Only one patient had a family history of stroke, but the cause was not indicated. The most common presenting symptom was hemorrhage (10 cases, 48%), followed by headache (5 cases, 24%), seizure (4 cases, 19%), and tinnitus (1 case, 5%). In one case, the AVM was incidentally discovered. The AVM locations were intraparenchymal within the brain in 20 cases, and dural-based in 1 case. When intraparenchymal, most AVMs were intracerebral (frontal lobe: 8 cases, parietal lobe: 5 cases, temporal lobe: 4 cases, occipital lobe: 2 cases). One case was cerebellar. AVM size was reported in 20/21 cases. The average AVM size was 3.3 cm (median: 3 cm, range: 1.7-9.0 cm).

KRAS mutation analysis revealed mutations in 6/21 cases (28.5%). Five of the 6 mutated cases involved p.G12V mutations, and 1 involved a p.G12C mutation. The *KRAS*-mutant group contained 4 females and 2 males, and the average patient age was 28 years (median: 24 years, range: 9-65 years). Presenting symptoms for the AVMs in the *KRAS*-mutant group included 4 cases with hemorrhage and 2 with headaches. All 6 of the *KRAS*-mutant cases were intracerebral (frontal lobe: 2 cases, temporal lobe: 2 cases, parietal lobe: 1 case, occipital lobe: 1 case). The average AVM size in the *KRAS*-mutant group was 3.9 cm. The non-mutant group contained 8 females and 7 males, and the average age was 34 years (median: 34 years, range: 11-57 years).\

Presenting symptoms for the AVMs in the non-mutant group included 6 cases with hemorrhage, 4 with seizures, 3 with headaches, 1 with tinnitus, and 1 was incidentally

discovered. The average AVM size in the non-mutant group was 3.1 cm (*n*=14). There was no

significant difference between *KRAS*-mutant and non-mutant groups regarding age (p=0.546),

AVM size (p=0.522), or gender (p=0.659). The patients with the aforementioned family history

of stroke and personal history of a vascular malformation in the parotid gland were in the non-

mutant group.

Examination of H&E-stained slides revealed similar diagnostic histologic features

between *KRAS*-mutant and non-mutant groups (**Figure 1**). Mitotic figures were not readily

identified in either group. Examination of Verhoeff-van Gieson-stained slides revealed that the

average ratio of AVM vessels containing completely or partially circumferential elastic laminae

to those that do not was 0.42 overall (range 0.25-0.75). The average ratio for *KRAS*-mutant

AVMs was 0.46 (range: 0.25-0.75), compared to 0.41 (range: 0.25-0.67) in non-mutant AVMs.

Ki-67 immunohistochemical stains performed on sections from each case revealed labelling that

was <1% in all cases, whether *KRAS*-mutant or non-mutant, in both the endothelial cells and

smooth muscle cells of the AVM vessels (**Figure 2**).

DISCUSSION

Brain AVMs are a common cause of intracranial hemorrhage. In addition to clinical

presentations relating to hemorrhage, brain AVMs can also have presentations including seizures

or headaches, while others (at least 15%) are asymptomatic[3]. Most AVMs are presumably

sporadic, but there are rare familial cases and AVMs also occur in a number of congenital

syndromes[4-7]. For example, there is a 4-13% prevalence of brain AVMs in patients with

hereditary hemorrhagic telangiectasia[15, 16]. Recently, activating *KRAS* mutations were noted

in 62.5% of 72 brain AVMs, but in none of 21 paired blood samples[8]. The *KRAS* mutations

reported, in order of frequency, were p.G12D, p.G12V, and p.Q61H. In this study, we

investigated *KRAS* mutations in brain AVMs and compared basic clinical and histologic information to assess for distinguishing features between *KRAS*-mutant and non-mutant AVMs.

Of 21 brain AVM cases in our cohort, 6 (28.5%) contained *KRAS* mutations by PCR. We thus demonstrate that a subset of brain AVMs contain *KRAS* mutations and confirm that *KRAS* mutations should be considered amongst possible mutations in AVMs that may represent key genetic drivers in their development. Other mutations reported in vascular malformations include somatic mutations in the RAS-MAPK pathway, which have been noted in brain AVMs, and mutations in the PI3K pathway[17-21]. Interestingly, *KRAS* is mostly implicated in tumorigenesis and cancer, where mutations promote unregulated activation of growth-promoting signal transduction pathways resulting in cell transformation and genomic instability [9-12]. The presence of *KRAS* mutations in brain AVMs thus raises the question of whether they may represent a neoplastic process rather than a malformative one. However, though activating *KRAS* mutations are usually affiliated with malignancy, brain AVMs are not known to have malignant potential. Also worth noting is that there may be different roles of *KRAS* mutations in the contexts of different disease processes. For example, endometriosis is essentially not associated with malignancy, but about one quarter of deep infiltrating endometriotic lesions have been reported to harbor driver mutations associated with cancer, including *KRAS* mutations [22]. Substantially more research is necessary in order to reach an understanding of cancer-associated driver mutations in the setting of benign, and even potentially non-neoplastic disease processes. *KRAS* mutations in brain AVMs may also have interesting implications with regard to therapy, as our knowledge of *RAS* oncogenes and potentially targetable, *RAS*-associated proteins and processes grows and therapies are put into clinical use[23, 24].

The most common *KRAS* mutation in our cohort was p.G12V, and no cases harbored a p.G12D mutation. This is in contrast to a previous report that detected p.G12D as the most common *KRAS* mutation in brain AVMs, followed by p.G12V[8]. In one case, our study also detected a p.G12C mutation in a brain AVM, and this has not been previously reported. This finding is of interest considering that *KRAS* p.G12C mutations have been recently identified as potentially targetable in cancer therapy and p.G12C inhibitors are currently being investigated.[25-28]

Clinical information in our cohort revealed no distinguishing features between *KRAS*-mutant and non-mutant groups. No patients had a history of multiple brain AVMs. Only one patient, a child with an AVM in the non-mutant group, had an additional vascular malformation outside of the brain. There were no documented family histories of intra- or extracranial vascular malformations. Presenting symptoms between the groups were similar, but none in the *KRAS*-mutant group presented with seizures. The average age in the *KRAS*-mutant group (28 years) was lower than the non-mutant group (34 years), but the oldest patient in the study was in the *KRAS*-mutant group, and the age difference was not statistically significant. The average AVM size was larger in the *KRAS*-mutant group (3.9 cm) than the non-mutant group (3.1 cm), but this also was not statistically significant. Additional studies with larger cohorts are necessary to definitively determine if there are substantive differences in patient age and AVM size between *KRAS*-mutant and non-mutant cases. Finally, conventional histology for brain AVMs was observed in all cases of our study and histologic characteristics, including proportions of elastin-containing vessels and evidence of proliferative activity, were equally distributed and similarly present in all brain AVMs regardless of *KRAS* status. Nikolaev et al. reported that *KRAS* mutations in the endothelial cells of brain AVMs in vitro initiated activation of the MAPK-ERK pathway [8].

They also concluded immunohistochemically that MAPK-ERK activation is a defining molecular feature of brain AVMs, with or without initiation by a *KRAS* mutation. Initiating events for AVMs without RAS mutation are uncertain. The result of our histologic investigation between *KRAS*-mutant and non-mutant AVMs likely indicates that the histology of brain AVMs is not specific to activating *KRAS* mutations. Instead, it may indicate that the histology of brain AVMs is a reflection of MAPK-ERK activation in general, regardless of the initiating event. This suggests that the presence of *KRAS* mutations within AVMs may be of little clinical or pathologic importance.

The frequency of *KRAS* mutations in brain AVMS noted in our study is lower than previously reported. There may be several reasons for this discrepancy. The first may be divergence in methodology. Our study implemented a therascreen *KRAS* RGQ PCR Kit, a FDA approved real-time PCR assay that detects 7 mutations in codons 12 and 13 of exon 2 of the *KRAS* gene. The estimated limit of detection (analytical sensitivity) of the *KRAS* assay in our laboratory is 1%. The original publication by Nikolaev et al. used droplet digital PCR, and established a minimum of 0.5% fractional abundance for a positive sample [8]. Discrepancy may also be due to difference in materials. Our study utilized only FFPE for real-time PCR testing, while the previous study utilized both FFPE and fresh frozen tissues for genetic anlaysis [8]. However, a large analysis of DNA quality from FFPE tissues was published by Einaga et al [29], and the results showed that the DNA extracted from FFPE tissue was sufficient for sequencing based tests.

Tissue sampling may also have contributed to discrepancy: brain AVMs contain numerous cell types and, as previously reported, it is endothelial cells that contain *KRAS* mutations in brain AVMs [8]. It is possible that mutations were undetected in some cases owing

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to undersampling of endothelial cells relative to other AVM components. Finally, there may be some discrepancy as a result of a potential difference in our patient population. Further studies would be necessary to assess the true frequency of *KRAS* mutations in brain AVMs,

In conclusion, we demonstrate that approximately one third of brain AVMs harbor *KRAS* mutations that may be among key genetic drivers behind their development. *KRAS* p.G12V was the most common *KRAS* mutation identified in our study. We also demonstrate the first reported instance of a *KRAS* p.G12C mutation in a brain AVM. The mean age of patients with *KRAS*-mutant AVMs was slightly lower than in the non-mutant group, and the mean size larger. In this study, all observed histologic criteria of brain AVMs were equally distributed and similarly present in all AVMs, regardless of *KRAS* status. This indicates that the clinical features and histology of brain AVMs are not specific to activating *KRAS* mutations, and therefore that *KRAS* mutations in brain AVMS may be of little clinical or pathologic importance.

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Figure Legends

Figure 1. Photomicrographs of brain arteriovenous malformations (AVMs), 100x magnification. **A-C**: non-mutant AVMs, **D-F**: *KRAS*-mutant AVMs. All AVMs displayed the conventional histology of mixed arterial and venous channels, and 'arterialized veins' embedded in gliotic brain parenchyma, and were occasionally noted with hemorrhage (B,F) and inflammatory infiltrates (arrow) (E). *KRAS*-mutant and non-mutant AVMS were not distinguishable histologically.

Figure 2. Photomicrographs of brain arteriovenous malformations (AVMs), 100x magnification. **A-C** non-mutant AVM case, **D-F**: *KRAS*-mutant AVM case. Comparison of Hematoxylin & Eosin (A and D), Verhoeff-van Gieson stains (B and E), and Ki-67 immunohistochemical stains (C and F). Regardless of mutation status, all AVMs displayed the conventional histology of mixed arterial and venous channels, and 'arterialized veins', evidenced further by discontinuities in the elastic lamina as demonstrated by Verhoeff-van Gieson staining. Also, both KRAS-mutant and non-mutant AVMs all similarly displayed little to no evidence of proliferative activity via Ki-67 immunohistochemical staining, with stains essentially being negative in all components of the lesions (proliferative index of <1%). The foci of brown discoloration in photomicrograph F are hemosiderin deposits.

Table 1. Arteriovenous malformation of the brain: individual case data

Case	Age	Sex	Presentation	AVM Location	AVM Side	AVM Size (cm)	*KRAS* status
1	24	Female	Seizure	Frontal lobe	Left	4.9	Wild-type
2	27	Female	Hemorrhage	Frontal lobe	Left	3.0	Wild-type
3	38	Male	Hemorrhage	Temporal lobe	Left	5.7	Wild-type
4	9	Female	Hemorrhage	Temporal lobe	Left	3.5	G12V
5	65	Male	Hemorrhage	Parietal lobe	Right	9.0	G12V
6	54	Female	Hemorrhage	Temporal lobe	Right	3.5	Wild-type
7	30	Female	Headache	Frontal lobe	Left	3.0	G12V
8	57	Male	Hemorrhage	Frontal lobe	Left	2.5	Wild-type
9	34	Male	Incidental	Parietal lobe	Right	1.7	Wild-type
10	13	Female	Hemorrhage	Frontal lobe	Right	4.1	G12V
11	32	Female	Hemorrhage	Occipital lobe	Left	2.0	G12C
12	18	Male	Headache	Parietal lobe	Right	1.7	G12V
13	55	Female	Seizure	Temporal lobe	Right	2.3	Wild-type
14	34	Male	Headache	Frontal lobe	Left	2.5	Wild-type
15	20	Female	Hemorrhage	Cerebellum	Midline	3.0	Wild-type
16	16	Female	Headache	Parietal lobe	Left	4.4	Wild-type
17	18	Male	Hemorrhage	Occipital lobe	Right	2.6	Wild-type
18	40	Male	Seizure	Frontal lobe	Left	2.5	Wild-type
19	11	Female	Headache	Frontal lobe	Left	2.0	Wild-type
20	40	Female	Seizure	Parietal lobe	Left	3.0	Wild-type
21	36	Male	Tinnitus	Dura	Midline	Not listed	Wild-type

Highlights

- Most brain arteriovenous malformations are presumably sporadic, but rare familial cases have been reported.

- *KRAS* mutations occur in almost one third of brain arteriovenous malformations (AVMs).

- All observed clinical and histologic data of brain AVMs were equally distributed and similarly present in all AVMs, regardless of *KRAS* status.
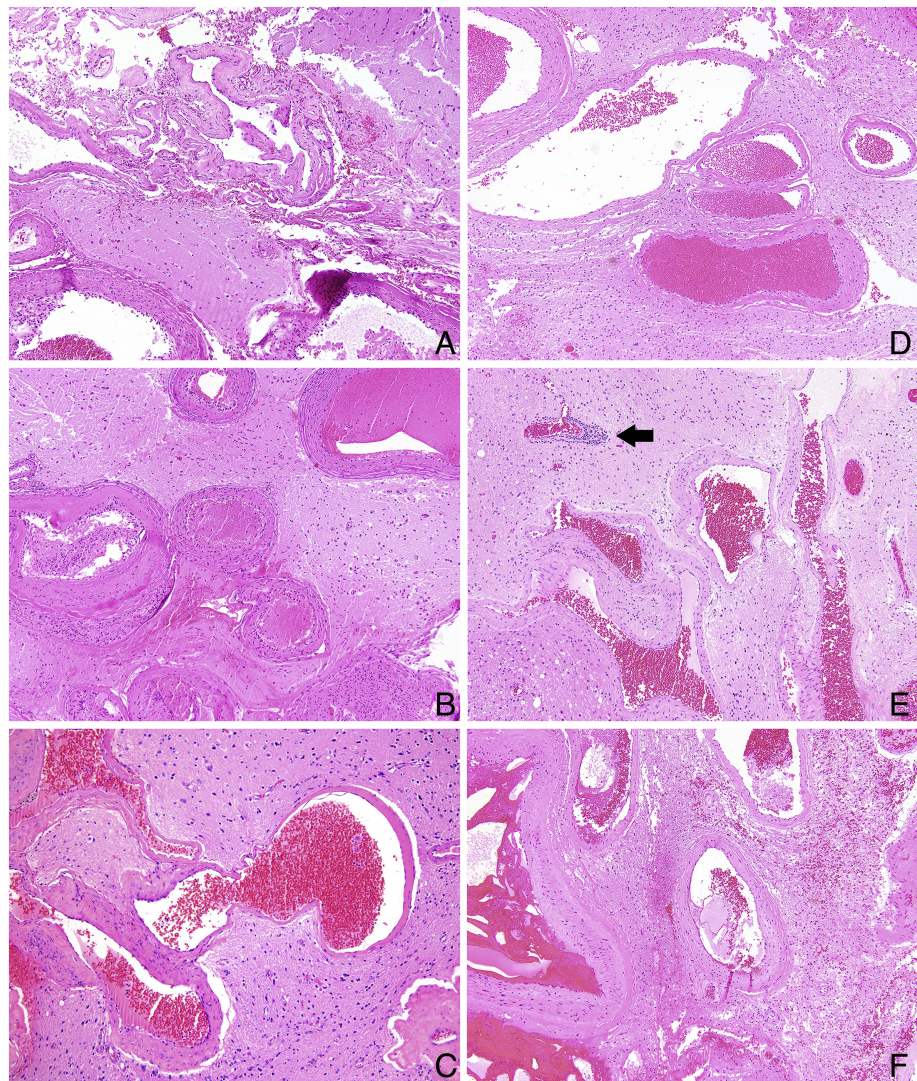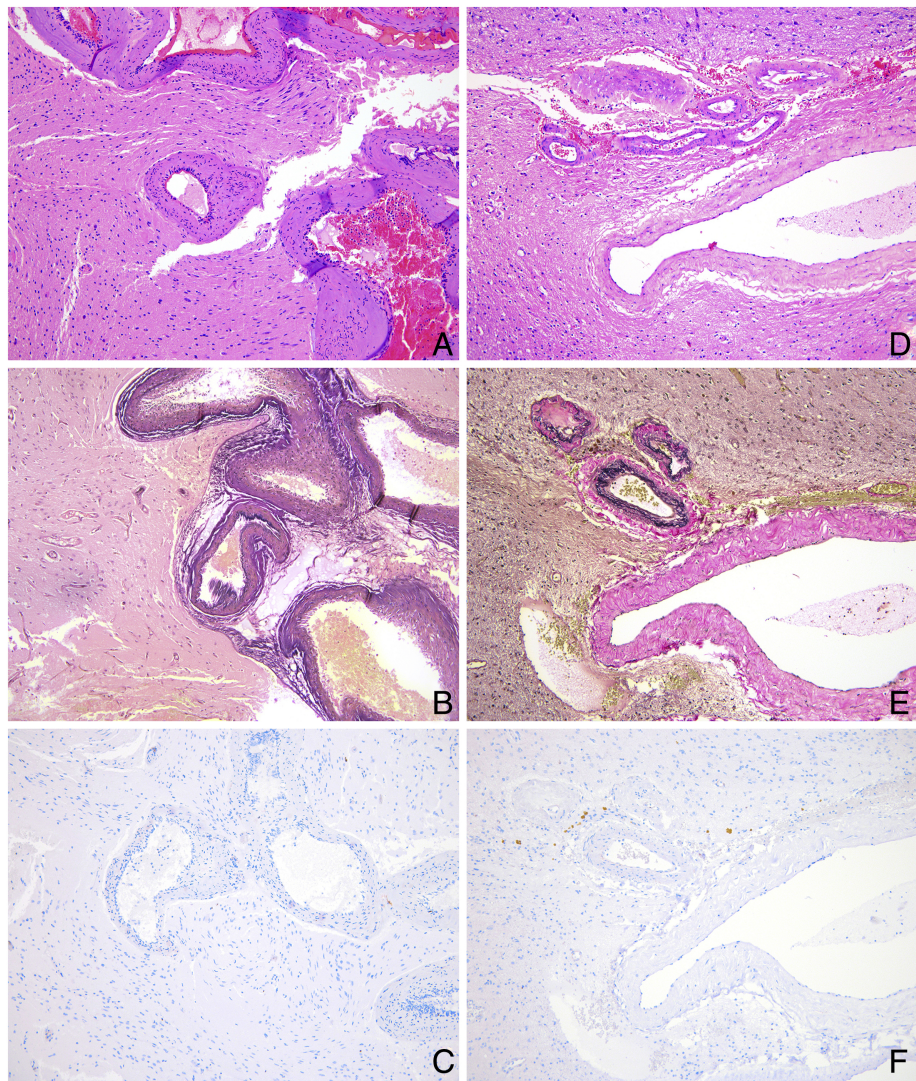
Figure 1

Figure 2