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Differential Gene Expression in Relation to the Clinical Characteristics of Human Brain Arteriovenous Malformations

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Abstract

Arteriovenous malformations (AVMs) of the central nervous system are considered as congenital disorders. They are composed of abnormally developed dilated arteries and veins and are characterized microscopically by the absence of a capillary network. We previously reported DNA fragmentation and increased expression of apoptosis-related factors in AVM lesions. In this article, we used microarray analysis to examine differential gene expression in relation to clinical manifestations in 11 AVM samples from Japanese patients. We categorized the genes with altered expression into four groups: death-related, neuron-related, inflammation-related, and other. The death-related differentially expressed genes were *MMP9*, *LIF*, *SOD2*, *BCL2A1*, *MMP12*, and *HSPA6*. The neuron-related genes were *NPY*, *S100A9*, *NeuroD2*, *S100Abeta*, *CAMK2A*, *SYNPR*, *CHRM2*, and *CAMKV*. The inflammation-related genes were *PTX3*, *IL8*, *IL6*, *CXCL10*, *GBP1*, *CHRM3*, *CXCL1*, *IL1R2*, *CCL18*, and *CCL13*. In addition, we compared gene expression in those with or without clinical characteristics including deep drainer, embolization, and high-flow nidus. We identified a small number of genes. Using these microarray data we are able to generate and test new hypotheses to explore AVM pathophysiology. Microarray analysis is a useful technique to study clinical specimens from patients with brain vascular malformations.

Key words: arteriovenous malformations, DNA microarray, clinical characteristics

Introduction

Arteriovenous malformations (AVMs) of the central nervous system are generally considered as congenital disorders that result from aberrant differentiation of the mesoderm during embryonic development. AVMs are composed of abnormally developed dilated arteries and veins and are characterized microscopically by the absence of a capillary network.[1–4] Although many studies have addressed the epidemiological characteristics, natural history, radiological features, and clinical behavior of AVMs, less is known about the molecular properties of these lesions.[1–4] Recent studies have revealed abnormal expression of angiogenic growth factors and their receptors compared with that in normal brain tissue.[5–8] Moreover, we have reported that AVM lesions display DNA fragmentation and increased expression of apoptosis-related factors.[9–11] In this study, we examined differential gene expression in AVMs and analyzed this expression in relation to clinical manifestations in Japanese patients.

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Materials and Methods

I. Patients

Eleven specimens from patients with cerebral AVMs were used in this study. All samples were obtained during surgery and were snap-frozen in liquid nitrogen. The relevant clinical and lesion features of the cases are summarized in Table 1.

II. Preparation of RNA

RNA was isolated from the specimens which is nudus including brain parenchyma as follows. Briefly, RNA*later*® (Life Technologies Inc., Carlsbad, California, USA) was added at a volume of 1 ml/100 mg sample. The samples were thawed and then homogenized

Table 1 Clinical summary of the patients

Case	Age	Sex	Hemorrhage	High flow	Deep	Embolization	Seizure	Size	S-M grade	Location
1	60	M	No	Yes	No	No	No	3 cm	2	Occipital
2	2	F	Yes	Yes	Yes	No	No	5 cm	4	Frontal
3	28	F	No	No	No	No	Yes	2 cm	1	Temporal
4	32	M	No	Yes	No	Yes	Yes	2 cm	1	Frontal
5	49	M	Yes	Yes	Yes	No	No	3 cm	2	Frontal
6	25	M	Yes	Yes	No	No	No	2 cm	2	Occipital
7	28	M	No	Yes	No	No	Yes	4 cm	2	Frontal
8	17	F	No	No	Yes	Yes	No	5 cm	4	Cerebellum
9	29	F	Yes (op)	Yes	No	Yes	No	4 cm	2	Temporooccipital
10	45	M	Yes	Yes	No	No	No	3 cm	2	Parietal
11	38	M	No	Yes	No	No	No	2 cm	1	Parietal

F: female, M: male, op: intraoperative hemorrhage, S-M: Spetzler and Martin.

three times for 20 sec on ice. After the addition of 0.1 vol 1-bromo-3-chloropropane, the homogenate was vortexed for 15 sec and incubated on ice for 1 hr. After centrifugation, the upper aqueous phase was transferred to a new tube and a half volume of isopropanol was added. The solution was then mixed and incubated on ice for 1 hr. After centrifugation, the supernatant was removed. The RNA pellet was washed with 80% ethanol and resuspended in diethylpyrocarbonate-treated water. The RNA was affinity column-purified using an RNeasy Mini Kit (Qiagen Inc., Valencia, California, USA) according to the manufacturer's protocol. Control RNA was extracted from a middle cerebral artery and a cortical tissue sample from a Caucasian male.

III. Microarray analysis

Microarray analysis was conducted by Hokkaido System Science Co. Ltd. (Sapporo). Total RNA was extracted from three biological replicates of each sample, and then it was used for cRNA synthesis. The resulting cRNA was subsequently labeled with Cyanin3 using a Quick Amp Labeling Kit (Agilent Technologies Inc., Santa Clara, California, USA), and purified using RNeasy mini spin columns (Qiagen) to generate the cRNA target solution. The cRNA target solution was then hybridized to the microarray (Arabidopsis Oligo DNA microarray ver. 4.0; Agilent Technologies). After washing and air-drying, the slide was scanned at a resolution of 5 μm using a microarray scanner (Agilent Technologies). The digitalized data were imported into software (GeneSpring GX 10; Agilent Technologies) and normalized to shift to the 75th percentile. The following flagged features were cut off: features that were not positive and significant, and features that were not above background levels. After filtering for flags, 32 348 probes remained. On the microarray, some genes are represented by several oligonucleotides that have distinct 60-mer sequences from different regions within the same gene.

Results

Tables 2 and 3 indicate the genes that displayed an absolute fold change of at least ± 300. We categorized these genes into four groups: death-related, neuron-related, inflammation-related, and others. The differentially expressed death-related genes were *MMP9*, *LIF*, *SOD2*, *BCL2A1*, *MMP12*, and *HSPA6*. The neuron-related genes were *NPY*, *S100A9*, *NeuroD2*, *S100Abeta*, *CAMK2A*, *SYNPR*, *CHRM2*, and *CAMKV*. The inflammation-related genes were *PTX3*, *IL8*, *IL6*, *CXCL10*, *GBP1*, *CHRM3*, *CXCL1*, *IL1R2*, *CCL18*, and *CCL13*. In addition, we classified significantly changed genes based on biological process and molecular function (Fig. 1).

Next, we analyzed gene expression in relation to clinical characteristics. First, we analyzed gene expression in the samples that were or were not from deep-draining veins. We identified 32 genes that showed greater than 10-fold change in deep-draining samples (Table 4). Among them, *FGF9*, which is an angiogenesis-related gene, was upregulated. We next compared gene expression in those with or without preoperative embolization, and found 21 genes that showed a greater than 10-fold change in those with embolization (Table 5). Among them, *PTX3*, *MMP3*, and *GDNF* were downregulated in the samples with preoperative embolization. When we compared expression in the samples with or without a high-flow nidus, we identified 40 genes with a greater

Table 2 Genes with altered expression in cerebral arteriovenous malformation Part 1

ProbeName	Regulation	Common name	Category	Description
A_23_P166848	Up	LTF	O	Homo sapiens lactotransferrin (LTF), mRNA
A_23_P40174	Up	MMP9	D	Homo sapiens matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) (MMP9), mRNA
A_23_P207520	Up	COL1A1	O	Homo sapiens mRNA for prepro-alpha1(I) collagen
A_23_P212914	Up	RUFY3	O	Homo sapiens RUN and FYVE domain containing 3 (RUFY3), transcript variant 1, mRNA
A_23_P121064	Up	PTX3	I	Homo sapiens pentraxin-related gene, rapidly induced by IL-1 beta (PTX3), mRNA
A_24_P122137	Up	LIF	D	Homo sapiens leukemia inhibitory factor (cholinergic differentiation factor) (LIF), mRNA
A_23_P53137	Up	HBG1	O	Homo sapiens hemoglobin, gamma A (HBG1), mRNA
A_32_P87013	Up	IL8	I	Homo sapiens interleukin 8 (IL8), mRNA
A_32_P70158	Up	LILRB3	O	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3 (LILRB3), transcript variant 2, mRNA
A_23_P142533	Up	COL3A1	O	Homo sapiens collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant) (COL3A1), mRNA
A_24_P24371	Up	ENST00000390543	O	Immunoglobulin heavy chain C gene segment [Source: IMGT/GENE_DB; Acc: IGHG4]
A_23_P71037	Up	IL6	A, I	Homo sapiens interleukin 6 (interferon, beta 2) (IL6), mRNA
A_23_P81898	Up	UBD	O	Homo sapiens ubiquitin D (UBD), mRNA
A_23_P324384	Up	RPS4Y2	O	Homo sapiens ribosomal protein S4, Y-linked 2 (RPS4Y2), mRNA
A_32_P385587	Up	ALAS2	O	Homo sapiens aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia) (ALAS2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA
A_24_P935819	Up	SOD2	D	Homo sapiens superoxide dismutase 2, mitochondrial, mRNA (cDNA clone MGC: 21350 IMAGE: 4184203), complete cds
A_24_P303091	Up	CXCL10	I	Homo sapiens chemokine (C-X-C motif) ligand 10 (CXCL10), mRNA
A_23_P106602	Up	CRISPLD2	O	Homo sapiens cysteine-rich secretory protein LCCL domain containing 2 (CRISPLD2), mRNA
A_23_P170233	Up	CSTA	O	Homo sapiens cystatin A (stefin A) (CSTA), mRNA
A_23_P158817	Up	IGH@	O	Homo sapiens cDNA FLJ27104 fis, clone SPL04981, highly similar to Ig gamma-2 chain C region
A_24_P169873	Up	ENST00000390539	O	Immunoglobulin heavy chain C gene segment [Source: IMGT/GENE_DB; Acc: IGHA2]
A_23_P62890	Up	GBP1	I	Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1), mRNA
A_32_P22654	Up	ALAS2	O	Homo sapiens aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia) (ALAS2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA
A_23_P33723	Up	CD163	O	Homo sapiens CD163 molecule (CD163), transcript variant 1, mRNA
A_32_P39440	Up	BC030813	O	Homo sapiens cDNA clone MGC: 22645 IMAGE: 4700961, complete cds
A_23_P23048	Up	S100A9	N	Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA

(*Continued*)

Table 2 (Continued)

ProbeName	Regulation	Common name	Category	Description
A_23_P256470	Down	NPY	N	Homo sapiens neuropeptide Y (NPY), mRNA
A_23_P205428	Down	FOXG1	O	Homo sapiens forkhead box G1B (FOXG1B), mRNA [NM_005249]
A_24_P817236	Down	ENST00000366569	O	Muscarinic acetylcholine receptor M3 [Source: Uniprot/SWISSPROT; Acc: P20309]
A_24_P142343	Down	HRNBP3	O	Homo sapiens hypothetical protein LOC146713 (HRNBP3), mRNA
A_24_P500584	Down	XIST	O	Homo sapiens X (inactive)-specific transcript (XIST) on chromosome X
A_32_P85360	Down	THC2770932	O	Unknown
A_24_P347319	Down	KCNC2	O	Homo sapiens potassium voltage-gated channel, Shaw-related subfamily, member 2 (KCNC2), transcript variant 1, mRNA
A_23_P401472	Down	CHRM3	I	Homo sapiens cholinergic receptor, muscarinic 3 (CHRM3), mRNA
A_32_P142818	Down	DLX1	O	Homo sapiens distal-less homeobox 1 (DLX1), transcript variant 1, mRNA
A_23_P67569	Down	PRG2	O	Homo sapiens plasticity-related gene 2 (PRG2), mRNA

A: angiogenesis, D: death, I: inflammation, N: neuron, O: others.

Table 3 Genes with altered expression in cerebral arteriovenous malformation Part 2

ProbeName	Regulation	Common name	Category	Description
A_24_P335092	Up	SAA1	O	Homo sapiens serum amyloid A1 (SAA1), transcript variant 1, mRNA
A_23_P43979	Up	M87790	O	Human (hybridoma H210) anti-hepatitis A immunoglobulin lambda chain variable region, constant region, complementarity-determining regions mRNA, complete cds
A_23_P434809	Up	S100A8	N	Homo sapiens S100 calcium binding protein A8 (S100A8), mRNA
A_23_P7144	Up	CXCL1	I	Homo sapiens chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (CXCL1), mRNA
A_23_P64539	Up	HBG1	O	Homo sapiens hemoglobin, gamma A (HBG1), mRNA
A_23_P79398	Up	IL1R2	I	Homo sapiens interleukin 1 receptor, type II (IL1R2), transcript variant 1, mRNA
A_23_P99515	Up	C13orf33	O	Homo sapiens chromosome 13 open reading frame 33 (C13orf33), mRNA
A_24_P357847	Up	BC030813	O	Homo sapiens cDNA clone MGC: 22645 IMAGE: 4700961, complete cds
A_23_P431388	Up	SPOCD1	O	Homo sapiens SPOC domain containing 1 (SPOCD1), mRNA
A_23_P152002	Up	BCL2A1	D	Homo sapiens BCL2-related protein A1 (BCL2A1), mRNA
A_23_P160286	Up	PRG4	O	Homo sapiens proteoglycan 4 (PRG4), mRNA
A_23_P90710	Up	DES	O	Homo sapiens desmin (DES), mRNA
A_23_P259071	Up	AREG	O	Homo sapiens amphiregulin (schwannoma-derived growth factor) (AREG), mRNA
A_32_P116488	Up	THC2677011	O	Unknown
A_24_P605563	Up	AY172962	O	Homo sapiens anti-rabies SOJB immunoglobulin lambda light chain mRNA, complete cds

(*Continued*)

Table 3 (Continued)

ProbeName	Regulation	Common name	Category	Description
A_23_P55270	Up	CCL18	I	Homo sapiens chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) (CCL18), mRNA
A_23_P4773	Up	LILRB5	O	Homo sapiens leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 5 (LILRB5), transcript variant 2, mRNA
A_23_P259314	Up	RPS4Y1	O	Homo sapiens ribosomal protein S4, Y-linked 1 (RPS4Y1), mRNA
A_23_P26965	Up	CCL13	I	Homo sapiens chemokine (C-C motif) ligand 13 (CCL13), mRNA
A_32_P192842	Up	BM129308	O	if20d02.x1 Melton Normalized Human Islet 4 N4-HIS 1 Homo sapiens cDNA clone IMAGE: 5677082 3', mRNA sequence
A_23_P340698	Up	MMP12	D	Homo sapiens matrix metallopeptidase 12 (macrophage elastase) (MMP12), mRNA
A_23_P114903	Up	HSPA6	D	Homo sapiens heat shock 70kDa protein 6 (HSP70B') (HSPA6), mRNA
A_32_P200144	Up	IGH@	O	Homo sapiens cDNA FLJ27104 fis, clone SPL04981, highly similar to Ig gamma-2 chain C region
A_32_P45738	Down	PGAM1	O	Homo sapiens phosphoglycerate mutase 1 (brain) (PGAM1), mRNA
A_23_P60130	Down	MAL2	O	Homo sapiens mal, T-cell differentiation protein 2 (MAL2), mRNA
A_23_P355377	Down	SLC12A5	O	Homo sapiens solute carrier family 12, (potassium-chloride transporter) member 5 (SLC12A5), mRNA
A_32_P25295	Down	NEUROD2	N	Homo sapiens neurogenic differentiation 2 (NEUROD2), mRNA
A_23_P2543	Down	CUX2	O	Homo sapiens cut-like 2 (Drosophila) (CUTL2), mRNA
A_24_P380311	Down	CAMK2A	N	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha (CAMK2A), transcript variant 1, mRNA
A_23_P302568	Down	SLC30A3	O	Homo sapiens solute carrier family 30 (zinc transporter), member 3 (SLC30A3), mRNA
A_23_P80718	Down	SYNPR	N	Homo sapiens synaptoporin (SYNPR), mRNA
A_23_P145606	Down	CHRM2	N	Homo sapiens cholinergic receptor, muscarinic 2 (CHRM2), transcript variant 1, mRNA
A_23_P29680	Down	CAMKV	N	Homo sapiens CaM kinase-like vesicle-associated (CAMKV), mRNA
A_23_P77731	Down	CRYM	O	Homo sapiens crystallin, mu (CRYM), transcript variant 1, mRNA
A_23_P252817	Down	SST	O	Homo sapiens somatostatin (SST), mRNA
A_23_P35725	Down	ANO3	O	Homo sapiens transmembrane protein 16C (TMEM16C), mRNA
A_23_P157926	Down	LINGO2	O	Homo sapiens leucine rich repeat and Ig domain containing 2 (LINGO2), mRNA
A_23_P408195	Down	TMEM155	O	Homo sapiens transmembrane protein 155 (TMEM155), mRNA

A: angiogenesis, D: death, I: inflammation, N: neuron, O: others.

than 10-fold change in samples with high flow (Table 6). Neuron-related genes, including *NPY* and *NeuroD*, were downregulated in high-flow AVMs.

Discussion

AVMs seem to have unique and relatively homogeneous molecular abnormalities that can be detected

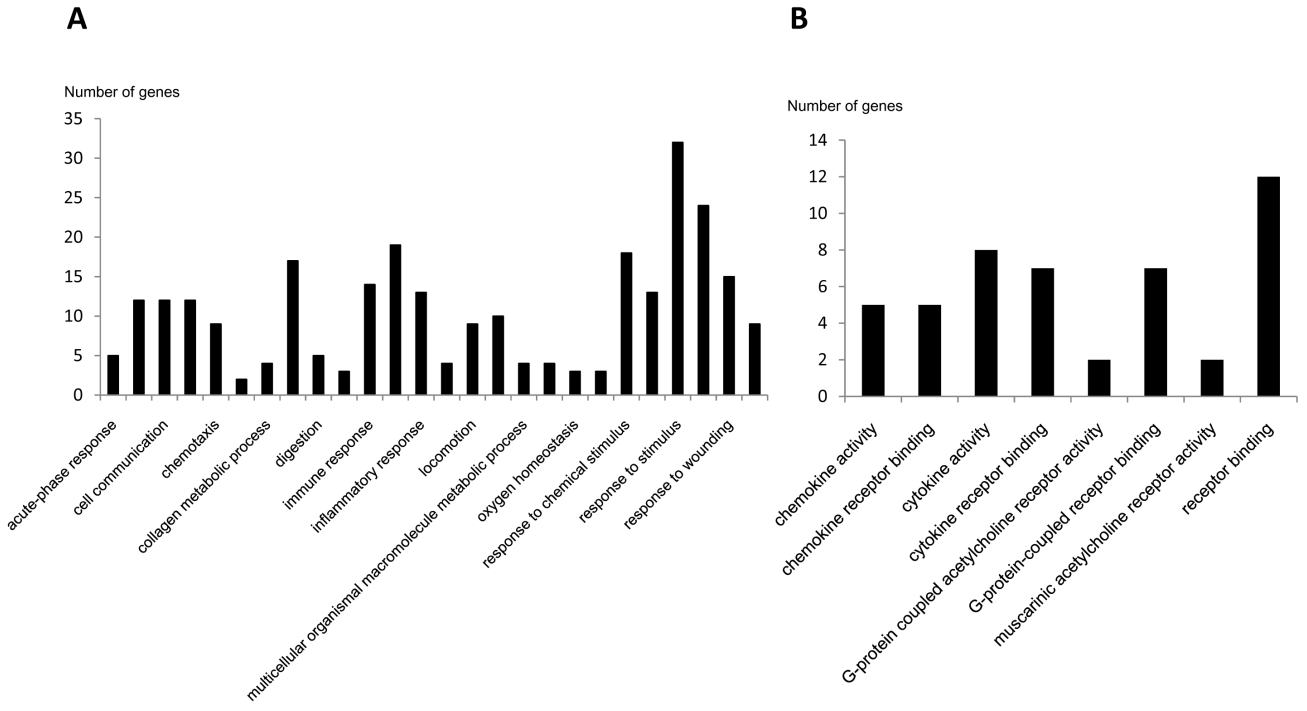

Fig. 1 Classified genes with significantly altered expression based on biological process (A) and molecular function (B).

at the mRNA and protein levels. Most studies have focused on the abnormal expression of vascular endothelial growth factor and its receptors[3,8,12–15] or angiogenesis or cell death-related factors and receptors. Moreover, we reported that the death receptor pathway and the NF-kappaB pathway were upregulated in AVMs.[9,11] These results indicate that dynamic vascular remodeling and neuronal death occur in and around the nidus of AVMs.[16–20] The majority of these studies, however, have focused on only one or a few genes or protein products. Here, using microarray analysis, we were able to dissect numerous molecular pathways that interact with or counteract each other within the same samples. Our findings were, in general, consistent with previously published findings, especially for genes showing a statistically significant difference between AVMs and controls.[3,8,12,15,21]

One previous study reported an increase in IL6 protein levels in AVM tissue. In addition, the GG genotype of the *IL6* 174G > C promoter polymorphism was associated with the clinical presentation of intracranial hemorrhage in AVMs.[8,13] As for *MMP9*, Hashimoto et al.[22] reported that AVM samples had higher levels of total MMP9, active MMP9, pro-MMP9, TIMP1, and TIMP3 than controls. In contrast, TIMP4 levels were higher in the control brain than in the AVM specimens. In addition, MMP9 was reported to be localized to the endothelial cell/peri-endothelial cell layer and infiltrating neutrophils of AVMs. Regarding IL1, we found that *IL1R2* was elevated in our AVM samples. Fontanella et al.[16] suggested that functional polymorphisms within the *IL1* complex gene are associated with AVMs and influence the clinical characteristics of the disease, supporting a role for proinflammatory cytokines in disease etiopathogenesis.[23] *IL1*β promoter polymorphisms were reported to be associated with AVM susceptibility and an increased risk of intracranial hemorrhage in the AVM clinical course.[16,23] These results suggest that the inflammatory pathways, including the IL1β cytokine, play an important role in intracranial hemorrhage. In previous studies, elevated IL6 was strongly associated with IL8 and MMP12, which were both elevated at the gene level in this study.[8,13] We and others have reported brain infiltration of various types of inflammatory cells in and around the nidus of AVMs.[10,24] We identified several chemokine genes to be elevated in AVMs; chemokines may be released by these infiltrating cells.[10,24] Previously we also showed reduced neuronal density around the nidus,[11] which may be related to our observed alterations in neuron-related genes. Our gene microarray data may help

Table 4 Clinical presentation and gene expression (deep-draining veins)

ProbeName	Fold change	Regulation	Common name	Category	Description
A_23_P24294	17.487488	Up	SLC17A6	O	Homo sapiens solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6 (SLC17A6), mRNA
A_32_P164593	12.236315	Up	ZMAT4	O	Homo sapiens zinc finger, matrin type 4 (ZMAT4), mRNA
A_23_P334308	10.814596	Up	MTUS2	O	Homo sapiens KIAA0774 (KIAA0774), transcript variant 1, mRNA
A_23_P2283	14.029393	Up	TAC3	O	Homo sapiens tachykinin 3 (neuromedin K, neurokinin beta) (TAC3), transcript variant 1, mRNA
A_23_P92860	12.137709	Up	CCNO	O	Homo sapiens cyclin U (CCNU), mRNA
A_24_P142343	21.59073	Up		O	Homo sapiens hypothetical protein LOC146713 (HRNBP3), mRNA
A_24_P25137	10.262987	Up	CHRM3	O	Homo sapiens cholinergic receptor, muscarinic 3 (CHRM3), mRNA
A_32_P166733	13.562277	Up	BU686948	O	UI-CF-DU1-ado-e-06-0-UI.s1 UI-CF-DU1 Homo sapiens cDNA clone UI-CF-DU1-ado-e-06-0-UI 3', mRNA sequence
A_23_P8981	12.29509	Up	STAR	O	Homo sapiens steroidogenic acute regulator (STAR), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA
A_23_P321846	15.988988	Up	KCNS1	O	Homo sapiens potassium voltage-gated channel, delayed-rectifier, subfamily S, member 1 (KCNS1), mRNA
A_24_P54900	12.008987	Up	LNX1	O	Homo sapiens ligand of numb-protein X 1 (LNX1), mRNA
A_24_P219474	14.443749	Up	MGAT5B	O	Homo sapiens mannosyl (alpha-1,6-)- glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, isozyme B (MGAT5B), transcript variant 1, mRNA
A_23_P144847	10.753042	Up	CDH12	O	Homo sapiens cadherin 12, type 2 (N-cadherin 2) (CDH12), mRNA
A_23_P318616	11.135667	Up	LRTM2	O	Homo sapiens leucine-rich repeats and transmembrane domains 2 (LRTM2), mRNA
A_32_P142818	10.828055	Up	DLX1	O	Homo sapiens distal-less homeobox 1 (DLX1), transcript variant 1, mRNA
A_23_P140858	11.060884	Up		O	Homo sapiens ataxin 2-binding protein 1 (A2BP1), transcript variant 4, mRNA
A_23_P2543	15.522975	Up	CUX2	O	Homo sapiens cut-like 2 (Drosophila) (CUTL2), mRNA
A_23_P337642	12.069429	Up	ATP2B3	O	Homo sapiens ATPase, Ca++ transporting, plasma membrane 3 (ATP2B3), transcript variant 1, mRNA
A_24_P380311	26.18467	Up	CAMK2A	N	Homo sapiens calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha (CAMK2A), transcript variant 1, mRNA
A_23_P13822	10.42801	Up	STYK1	O	Homo sapiens serine/threonine/tyrosine kinase 1 (STYK1), mRNA
A_23_P65918	11.070756	Up	ITPKA	O	Homo sapiens inositol 1,4,5-trisphosphate 3-kinase A (ITPKA), mRNA
A_23_P157027	11.949467	Up		O	Homo sapiens hypothetical protein LOC 285878, mRNA (cDNA clone IMAGE: 5299807)
A_23_P22723	12.559601	Up	ATP2B3	O	Homo sapiens ATPase, Ca++ transporting, plasma membrane 3 (ATP2B3), transcript variant 1, mRNA

(*Continued*)

Table 4 (Continued)

ProbeName	Fold change	Regulation	Common name	Category	Description
A_23_P132175	11.3289	Up	RTN4R	O	Homo sapiens reticulon 4 receptor (RTN4R), mRNA
A_23_P79968	10.7763815	Up	PCSK2	O	Homo sapiens proprotein convertase subtilisin/kexin type 2 (PCSK2), mRNA
A_23_P105803	11.011639	Up	FGF9	A	Homo sapiens fibroblast growth factor 9 (glia-activating factor) (FGF9), mRNA
A_23_P53137	11.880983	Down	HBG1	O	Homo sapiens hemoglobin, gamma A (HBG1), mRNA
A_32_P385587	15.667379	Down	ALAS2	O	Homo sapiens aminolevulinate, delta-, synthase 2 (sideroblastic/hypochromic anemia) (ALAS2), nuclear gene encoding mitochondrial protein, transcript variant 1, mRNA
A_23_P121596	19.073265	Down	PPBP	I	Homo sapiens pro-platelet basic protein (chemokine [C-X-C motif] ligand 7) (PPBP), mRNA
A_32_P168342	10.181584	Down	C6orf25	O	G6b protein precursor [Source: Uniprot/SWISSPROT; Acc: O95866]
A_23_P87346	14.329434	Down	HBD	O	Homo sapiens hemoglobin, delta (HBD), mRNA
A_24_P79403	13.201708	Down	PF4	O	Homo sapiens platelet factor 4 (chemokine [C-X-C motif] ligand 4) (PF4), mRNA

A: angiogenesis, D: death, I: inflammation, N: neuron, O: others.

us to establish further hypotheses for testing. For example, microarray data showed inflammation-related genes including IL-8 and IL-6. These observations may lead us to anti-inflammatory treatment against AVMs. To establish this hypothesis, further study is necessary to confirm that inflammation increase the risk of AVM rupture. In addition, MMP family including *MMP9*, *MMP12*, and *MMP3* changed. This observation may lead us to perform other further analysis using MMP inhibitors.

In this study, we analyze gene expression focusing on the neuron-, death-, angiogenesis-, and inflammation-related genes. Because we and others indicated the role of these pathways in cerebral AVMs.[5,9–14,16,21,23,24] Decreased expression of neuron-related genes indicate the loss of neurons in and around the nidus. Increased expressions of death-related genes indicate cellular death of neurons, infiltrating and vascular cells. In addition, increased expression of angiogenesis and inflammation-related genes may show upregulation of these events.

We also analyzed associations between gene expression and the clinical presentation or treatment of AVMs (the presence or absence of hemorrhage, deep-draining veins, embolization, and high-flow), focusing on the neuron-, death-, angiogenesis-, and inflammation-related genes. A deep-draining system may cause venous congestion, which can lead to neuronal loss. However, our data did not indicate neuronal loss because *FGF9*, which can induce angiogenesis, was upregulated. We focused on inflammation-related genes in relation to preoperative embolization, and demonstrated downregulation of several genes in embolized samples. This may indicate that these changes are not related to preoperative embolization but instead to the operative process itself (two of these samples had intraoperative hemorrhage). In the high-flow samples, neuron-related genes, including neuropeptide Y (*NPY*), synaptotagmin 1 (*SYT1*), neurogenic differentiation 2 (*NeuroD*), and ephrin B3 (*EFNB3*) were downregulated. This may indicate that neurons and neuronal networks were injured in high-flow AVMs, and may correlate with our previous finding of neuronal loss in the perinidal area.[11]

One of the limitations of this study, and of most previous studies, is the small sample size, which can lead to false-negative or false-positive results. Clinical samples may show significant variation in the levels of a specific gene or its product, which may reflect different stages and severity of the disease or simply interindividual variation. One more limitation of the study, during surgical process gene expression may be affected with local ischemia, inflammation, mechanical compression and coagulation. Microarray analysis on a large number of clinical specimens with a well-characterized clinical background is necessary to validate our findings. In addition, it should be noted that the correlation between gene expression and that of its

Table 5 Clinical presentation and gene expression (embolization)

ProbeName	Fold change	Regulation	Common name	Category	Description
A_23_P62857	12.33438	Down	A_23_P62857	O	PLA2G2A
A_23_P73526	13.889064	Down	CITED1	O	Homo sapiens Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 1 (CITED1), mRNA
A_23_P121064	32.46276	Down	PTX3	I	Homo sapiens pentraxin-related gene, rapidly induced by IL-1 beta (PTX3), mRNA
A_32_P107372	15.64205	Down	GBP1	I	Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1), mRNA
A_23_P78037	15.200374	Down	CCL7	I	Homo sapiens chemokine (C-C motif) ligand 7 (CCL7), mRNA
A_23_P161698	16.020575	Down	MMP3	D	Homo sapiens matrix metallopeptidase 3 (stromelysin 1, progelatinase) (MMP3), mRNA
A_32_P377880	13.725988	Down	GDNF	N	Glial cell line-derived neurotrophic factor precursor (Astrocyte- derived trophic factor 1) (ATF-1)
A_32_P5417	17.92484	Down	CA946373	O	CA946373 ni04a06.x1 Human lacrimal gland: ni Homo sapiens cDNA clone ni04a06 5', mRNA sequence
A_23_P62890	16.525377	Down	GBP1	O	Homo sapiens guanylate binding protein 1, interferon-inducible, 67kDa (GBP1), mRNA
A_23_P52067	12.040656	Down	GRHL3	O	Homo sapiens grainyhead-like 3 (Drosophila) (GRHL3), transcript variant 2, mRNA
A_24_P932887	45.99536	Down	SPOCD1	O	Homo sapiens cDNA FLJ39908 fis, clone SPLEN2017620
A_23_P63254	12.1611395	Down	SFN	O	Homo sapiens stratifin (SFN), mRNA
A_24_P335092	50.85907	Down	SAA1	O	Homo sapiens serum amyloid A1 (SAA1), transcript variant 1, mRNA
A_23_P336554	11.759418	Down	IL1RAP	I	Homo sapiens interleukin 1 receptor accessory protein (IL1RAP), transcript variant 2, mRNA
A_23_P431388	24.716013	Down	SPOCD1	O	Homo sapiens SPOC domain containing 1 (SPOCD1), mRNA
A_32_P15544	7.8432913	Down	PRIMA1	O	Homo sapiens proline rich membrane anchor 1 (PRIMA1), mRNA
A_24_P923854	15.754891	Down	AF113674	O	Homo sapiens clone FLB1727 PRO0398 mRNA, complete cds
A_23_P104073	11.630592	Down	S100A3	N	Homo sapiens S100 calcium binding protein A3 (S100A3), mRNA
A_32_P116488	11.215696	Down	THC2677011	O	Unknown
A_24_P379521	28.073503	Down	BM702245	O	UI-E-CQ1-aey-h-03-0-UI.r1 UI-E-CQ1 Homo sapiens cDNA clone UI-E-CQ1-aey-h-03-0-UI 5', mRNA sequence
A_23_P306203	20.476131	Down	SAA2	O	Homo sapiens serum amyloid A2 (SAA2), mRNA

A: angiogenesis, D: death, I: inflammation, N: neuron, O: others.

Table 6 Clinical presentation and gene expression (high-flow)

ProbeName	Fold change	Regulation	Common name	Category	Description
A_24_P933319	13.79898	Down	RAB3B	O	Ras-related protein Rab-3B [Source: Uniprot/SWISSPROT; Acc: P20337]
A_23_P415541	26.580494	Down	GPR26	O	Homo sapiens G protein-coupled receptor 26 (GPR26), mRNA
A_32_P51005	12.428625	Down	AL834342	O	Homo sapiens mRNA; cDNA DKFZp761P2314 (from clone DKFZp761P2314)
A_32_P66804	12.633398	Down	PTPRN2	O	Homo sapiens protein tyrosine phosphatase, receptor type, N polypeptide 2 (PTPRN2), transcript variant 1, mRNA
A_23_P256470	56.132885	Down	NPY	N	Homo sapiens neuropeptide Y (NPY), mRNA
A_32_P183367	17.20502	Down	BRUNOL4	O	PREDICTED: Homo sapiens bruno-like 4, RNA binding protein (Drosophila) (BRUNOL4), mRNA
A_32_P152195	11.834058	Down	STAC2	O	Homo sapiens SH3 and cysteine rich domain 2 (STAC2), mRNA
A_24_P307964	21.591301	Down	SOHLH1	O	Homo sapiens spermatogenesis and oogenesis specific basic helix-loop-helix 1 (SOHLH1), mRNA
A_32_P323	13.151337	Down	BC037323	O	Homo sapiens cDNA clone IMAGE: 5261489
A_32_P3476	16.78356	Down	RPRML	O	Homo sapiens reprimo-like (RPRML), mRNA
A_24_P393571	15.406029	Down	GDA	O	Homo sapiens guanine deaminase (GDA), mRNA
A_23_P392654	13.023042	Down	SPHKAP	O	Homo sapiens SPHK1 (sphingosine kinase type 1) interacting protein (SKIP), mRNA
A_24_P479551	14.176087	Down	UBE2QL1	O	Homo sapiens mRNA, clone: TH049G03
A_24_P944714	11.678925	Down	ENST00000381655	O	Probable phospholipid-transporting ATPase IB (EC 3.6.3.1) (ATPase class I type 8A member 2) (ML-1)
A_32_P197156	27.140347	Down	BI758260	O	603029911F1 NIH_MGC_114 Homo sapiens cDNA clone IMAGE:5200131 5', mRNA sequence [BI758260]
A_23_P162010	15.891171	Down	CCKBR	O	Homo sapiens cholecystokinin B receptor (CCKBR), mRNA
A_23_P10025	11.819316	Down	NELL2	O	Homo sapiens NEL-like 2 (chicken) (NELL2), mRNA
A_23_P36795	14.929889	Down	SYT1	N	Homo sapiens synaptotagmin I (SYT1), mRNA
A_23_P67569	21.238386	Down		O	Homo sapiens plasticity-related gene 2 (PRG2), mRNA
A_32_P25295	28.055656	Down	NEUROD2	N	Homo sapiens neurogenic differentiation 2 (NEUROD2), mRNA
A_24_P940006	11.538975	Down	EFNB3	A	Homo sapiens ephrin-B3 (EFNB3), mRNA
A_32_P84369	12.537511	Down	FAM153B	O	Homo sapiens hypothetical protein LOC202134 (LOC202134), mRNA
A_23_P429601	25.979113	Down	GALNTL5	O	Homo sapiens UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 5 (GALNTL5), mRNA
A_23_P215283	11.123473	Down	TAC1	O	Homo sapiens tachykinin, precursor 1 (TAC1), transcript variant beta, mRNA
A_23_P50928	14.404265	Down	C1QL2	O	Homo sapiens complement component 1, q subcomponent-like 2 (C1QL2), mRNA

(*Continued*)

Table 6 (Continued)

ProbeName	Fold change	Regulation	Common name	Category	Description
A_23_P60775	10.825105	Down	BRUNOL5	O	Homo sapiens bruno-like 5, RNA binding protein (Drosophila) (BRUNOL5), mRNA
A_23_P357207	13.065118	Down	MRAP2	O	Homo sapiens chromosome 6 open reading frame 117 (C6orf117), mRNA
A_23_P204885	11.051615	Down	PCDH20	O	Homo sapiens protocadherin 20 (PCDH20), mRNA
A_24_P548966	15.763181	Down	RAB3B	O	Ras-related protein Rab-3B [Source:Uniprot/SWISSPROT;Acc:P20337]
A_23_P92730	19.459343	Down	HSPB3	D	Homo sapiens heat shock 27kDa protein 3 (HSPB3), mRNA
A_23_P252817	20.574722	Down	SST	O	Homo sapiens somatostatin (SST), mRNA
A_23_P428485	10.119454	Down	SPHKAP	O	Homo sapiens SPHK1 (sphingosine kinase type 1) interacting protein (SKIP), mRNA
A_32_P45844	13.05868	Down	BX110856	O	BX110856 Soares adult brain N2b4HB55Y Homo sapiens cDNA clone IMAG-p998M09331, mRNA sequence
A_23_P368794	22.000626	Down	TCERG1L	O	Homo sapiens transcription elongation regulator 1-like (TCERG1L), mRNA
A_24_P266131	15.002172	Down	FSTL4	O	Homo sapiens follistatin-like 4 (FSTL4), mRNA
A_23_P100022	16.488022	Down	SV2B	O	Homo sapiens synaptic vesicle glycoprotein 2B (SV2B), mRNA
A_23_P324706	11.529245	Down	FAM153A	O	Homo sapiens mRNA for KIAA0752 protein, partial cds
A_23_P51019	13.003195	Down	SCN2A	O	Homo sapiens sodium channel, voltage-gated, type II, alpha subunit (SCN2A), transcript variant 1, mRNA
A_23_P408195	28.795895	Down	TMEM155	O	Homo sapiens transmembrane protein 155 (TMEM155), mRNA
A_24_P38290	10.143327	Down	TAC1	O	Homo sapiens tachykinin, precursor 1 (TAC1), transcript variant beta, mRNA

A: angiogenesis, D: death, I: inflammation, N: neuron, O: others.

protein product is extremely variable. Transcription efficiency, post-transcriptional modification, and protein metabolism can all independently affect gene and protein levels.

In conclusion, we examined gene expression in AVMs by microarray analysis. Using our data, we are able to generate and test new hypotheses to explore AVM pathophysiology. Microarray analysis is a useful technique to study clinical specimens from patients with brain vascular malformations.

Conflicts of Interest Disclosure

All authors have no conflicts of interest. In addition, authors who are members of The Japan Neurosurgical Society (JNS) state that all authors have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

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