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# RNA-Sequencing Highlights Inflammation and Impaired Integrity of the Vascular Wall in Brain Arteriovenous Malformations

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***Background and Purpose***—Interventional treatment of unruptured brain arteriovenous malformations (BAVMs) has become increasingly controversial. Because medical therapy is still lacking, we aimed to obtain insight into the disease mechanisms implicated in BAVMs and to identify potential targets for medical treatment to prevent rupture of a BAVM.

***Methods***—We used next-generation RNA sequencing to identify differential expression on a transcriptome-wide level comparing tissue samples of 12 BAVMs to 16 intracranial control arteries. We identified differentially expressed genes by negative binominal generalized log-linear regression (false discovery rate corrected $P<0.05$). We selected 10 genes for validation using droplet digital polymerase chain reaction. We performed functional pathway analysis accounting for potential gene-length bias, to establish enhancement of biological pathways involved in BAVMs. We further assessed which Gene Ontology terms were enriched.

***Results***—We found 736 upregulated genes in BAVMs including genes implicated in the cytoskeletal machinery and cell-migration and genes encoding for inflammatory cytokines and secretory products of neutrophils and macrophages. Furthermore, we found 498 genes downregulated including genes implicated in extracellular matrix composition, the binary angiopoietin-TIE system, and TGF (transforming growth factor)-β signaling. We confirmed the differential expression of top 10 ranked genes. Functional pathway analysis showed enrichment of the protein digestion and absorption pathway (false discovery rate-adjusted $P=1.70\times10^{-2}$). We identified 47 enriched Gene Ontology terms (false discovery rate-adjusted $P<0.05$) implicated in cytoskeleton network, cell-migration, endoplasmic reticulum, transmembrane transport, and extracellular matrix composition.

***Conclusions***—Our genome-wide RNA-sequencing study points to involvement of inflammatory mediators, loss of cerebrovascular quiescence, and impaired integrity of the vascular wall in the pathophysiology of BAVMs. Our study may lend support to potential receptivity of BAVMs to medical therapeutics, including those promoting vessel maturation, and anti-inflammatory and immune-modifying drugs. **(*Stroke*. 2020;51:268-274. DOI: 10.1161/STROKEAHA.119.025657.)**

**Key Words:** brain ■ cerebral hemorrhage ■ gene expression profiling ■ macrophages ■ polymerase chain reaction



Interventional treatment for (un)ruptured brain arteriovenous malformations (BAVMs), to prevent (re)hemorrhage, is not without risk of severe complications.[1] Treatment of BAVMs not (yet) ruptured has become increasingly controversial.[2] Furthermore, a subset of BAVMs is deemed unsuitable for any intervention. For these reasons, medical therapy to diminish or obliterate the risk of rupture of a BAVM is needed.

Somatic activating mutations in the RAS oncogenes *KRAS* and *BRAF* have recently been identified in endothelial cells (ECs) of sporadic BAVMs.[3,4] The molecular pathways leading

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to hemorrhage, however, remain largely to be elucidated. Evidence is mounting that BAVMs are dynamic lesions, including continuous remodeling of the vessel wall.[5] In addition, vascular walls of BAVMs show infiltration of inflammatory mediators.[6,7] Thus far, 4 relatively small microarray-based gene expression studies, ranging from 4 to 11 included BAVM tissue samples and with only up to 5 control samples have identified between 19 and 1781 differentially expressed genes. However, methodological issues, such as the use of different types of control samples, including superficial temporal arteries, cortical arteries, draining veins of BAVMs and cerebral cavernous malformations, preclude any unequivocal conclusions to be drawn.[8–11]

Next-generation RNA sequencing provides more accurate, sensitive, and reliable gene expression data than can be achieved with microarrays.[12] Elucidating genome-wide expression profiling of BAVMs may aid in paving the way for developing novel therapeutics. We, therefore, used next-generation RNA sequencing to identify differential expression on a transcriptome-wide level comparing BAVM tissue samples to control samples of intracranial arteries.

## Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Patients and Controls

We obtained BAVM tissue from 12 patients who underwent neurosurgical resection of their BAVM at the Department of Neurology and Neurosurgery of the University Medical Center Utrecht between 2010 and 2013. Patients diagnosed with a hereditary disorder, like hereditary hemorrhagic telangiectasia, were excluded. BAVM diagnosis was confirmed by digital subtracted angiography in all patients. Hemorrhage from the BAVM was the reason for surgical removal in 11 patients, whereas for one this reason was epilepsy. We obtained control samples of cortical intracranial arteries from 16 patients in whom temporal lobectomy was performed because of medically intractable seizures. In these control patients, a biopsy of an intracranial cortical artery of normal appearance was obtained from the resected brain tissue containing the epileptogenic focus. Immediately after resection, BAVM and control tissue samples were snap-frozen in liquid nitrogen and stored at −80°C until further usage. The institutional review board of the University Medical Center Utrecht approved the present study. Informed consent was obtained from all patients and controls for the use of clinical data, and the use of tissue from controls. Informed consent was waived for the use of BAVM tissue after surgical excision.

### Library Preparation and RNA Sequencing

Information regarding RNA isolation and quantity and quality of the extracted RNA of each sample is provided in the methods section of the online-only Data Supplement. Mean RNA quality score was 6.4 (range, 5.4–7.5) in the BAVM samples and 7.2 (range, 4.2–9.0) in the control samples.

The sequence libraries were generated using the TruSeq mRNA sample preparation kit from Illumina (San Diego) by use of the Sciclone NGS Liquid Handler (Perkin Elmer) according to the manufacturer's instructions. After extra purification of the libraries, we sequenced 9 picomoles of each obtained cDNA fragment library on an Illumina HiSeq2500 High Output Standard using default parameters (single read 1×100 bp) in random pools of 10 or 11 samples. On average, 6 979 252 reads were generated for the BAVM samples and 8 247 012 reads for the control samples.

Good quality of the data was ensured by showing on average Phred-scores exceeding the predefined minimum of 30 in over 90% of the reads of each position, equivalent to a base call accuracy of 99.9% (Figure I in the online-only Data Supplement). Quality control of the reads, alignment to the build 37 human reference genome, and subsequent gene annotation are described in detail in the online-only Data Supplement. The average alignment of the reads to the human reference genome (uniquely mapped reads) was 86% (range, 73%–90%).

### Differential Expression Analysis

We used the counts per gene of each sample obtained after alignment as input for subsequent differential expression analyses. To increase statistical power, we removed low count genes with <1 read per million in the samples of the condition with the lowest number of replicates (the BAVM group). Counts of each sample were normalized using the trimmed mean of M-values to remove systematic bias caused by technical variability between libraries.[13] To account for biological variation, we calculated common and tag-wise dispersion rates using the Cox-Reid profile-adjusted likelihood method. Figure II in the online-only Data Supplement shows the resulting principal component plot. Tag-wise dispersion was shrunken towards the mean to account for overdispersion, for which we set the degree of freedom at 5. We designed a negative binominal generalized log-linear regression model including age as covariate, which was fitted to the read counts for each gene for genewise statistical testing, followed by a likelihood ratio test. We considered genes with a 2-tailed $P<0.05$ after false discovery rate (FDR) correction according to Benjamini and Hochberg to be differentially expressed. Analyses were performed using the package edgeR (version 3.6.2)[13] in the R software (version 3.1.0; http://www.r-project.org/), the Bioconductor (version 2.14; http://www.bioconductor.org) and the supporting packages limma (version 3.20.2) and biomaRt (version 2.20.0).

### Internal Validation

From the top 10 ranked genes showing the strongest differential expression, we selected 6 genes for validation. In addition, we selected 4 genes for validation because of biological interest. We determined mRNA expression in the remaining of the extracted RNA using droplet digital polymerase chain reaction, which allows for a sensitive detection and accurate quantification of even low abundant transcripts. The online-only Data Supplement contains a detailed description of this procedure. An insufficient amount of RNA of the control samples was left after RNA sequencing. Consequently, RNA of 14 control samples was available for validating *CHIT1*, 12 were left for *CFAP43*, 10 for *LTF*, 6 for *SLC47A2*, and 5 for *ZMYND10*. We used the Mann-Whitney *U* test to assess the relative differences in gene expression between BAVM and control samples and considered genes with a Bonferroni corrected $P<0.05$ as differentially expressed.

### Functional Pathway Analysis

To identify enriched biological functional pathways, we analyzed which Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) functional pathways, and Gene Ontology (GO) terms were enriched in our data set. We used the package GOseq (version 1.16.2) to account for potential gene-length bias.[14] Gene-length was obtained from the build 37 human reference genome. In case of one or more known splice variants the median length of the transcripts was used. For these analyses, we used genes identified as differentially expressed with an FDR-adjusted $P<0.05$ as input and used FDR correction ($P<0.05$) to assess significantly enriched pathways. Further trimming of enriched GO terms (FDR-adjusted $P<0.05$), to get rid of largely redundant terms, is described in the online-only Data Supplement. We differentiated GO terms into biological processes, cellular components, and molecular functions.

### Genes and Signaling Pathways Previously Implicated in BAVMs

We investigated whether causal genes for BAVMs (*KRAS*, *BRAF*, *ENG*, *ACVRL1* [*ALK1*], *SMAD4*, *RASA1*, and *EPHB4*) showed

differential expression. Given the similarities between sporadic BAVMs and those in hereditary hemorrhagic telangiectasia in both appearance and clinical behavior, we also investigated whether genes of the pleiotropic canonical TGF (transforming growth factor)-β/BMP (bone morphogenetic protein) signaling pathway showed differential expression as perturbed TGF-β/BMP signaling in hereditary hemorrhagic telangiectasia is key.[15] We also investigated whether genes implicated in Notch-1 signaling[16] and vascular sprouting, including VEGF (vascular endothelial growth factor; receptors),[17] and the binary angiopoietin-TIE system[18] showed differential expression because a role in BAVM pathophysiology has previously been ascribed to these angiogenic factors.

## Results

### Patient Characteristics

Of the 12 patients with a BAVM, 11 were men and their mean age at the time of surgery was 43.9 years (SD 17.5; range, 16–69). Their clinical and radiological characteristics are presented in Table 1. The median time interval between hemorrhage and surgery was 8.7 months (range, 0.5–22.3; 11 patients). Nine of 16 controls were men, and their mean age was 29.9 years (SD 11.8; range 16–50).

### Differential Expression Analysis

The expression of 1234 genes differed between BAVM specimens and intracranial control arteries after FDR correction ($P<0.05$; Figures 1 and 2; Table II in the online-only Data Supplement). Of these genes, 736 (59.6%) were upregulated in BAVMs, whereas 498 (40.4%) genes were downregulated. The median log-fold change of the genes showing differential expression was 1.46 (range, 0.45–5.62).

The 10 genes showing the strongest differential expression between BAVMs and intracranial control arteries are presented in Table 2. Among the top 10 ranked genes were 2 upregulated genes (*MYRF* and *MBP*) involved in axon myelination, and 2 upregulated genes implicated in inflammation, *CHIT1* and *LTF*. *CHIT1* encodes for chitotriosidase, which is produced by activated macrophages. *LTF* encodes for lactotransferrin, which is released by the secretory granules of neutrophils. The downregulated gene *DDR2* encodes for a tyrosine kinase receptor involved in the regulation of cell growth, differentiation, and metabolism. Other top 10 genes with known function included the upregulated genes *ZMYND10*, *CFAP43*, and *C9orf117* that are implicated in cytoskeletal microtubular motor activity, including axonemal dyneins, and the upregulated gene *SLC47A2* encoding for a transporter protein involved in extrusion of (toxic) metabolites but possibly also in motile cilia.

### Internal Validation

We confirmed the differential expression of 6 genes (*ZMYND10*, *DDR2*, *CFAP43*, *SLC47A2*, *CHIT1*, and *LTF*) ranked among the 10 strongest differentially expressed genes by droplet digital polymerase chain reaction (Table 2). The differential expression of the 4 additional genes, selected out of biological interest, could be confirmed for *IL1A*, but not for *ROCK1*, *COL5A2*, and *SMAD4* (Table III in the online-only Data Supplement).

**Table 1. Clinical and Radiological Characteristics of the Patients With a BAVM**

| BAVM | Sex | Age, y | Clinical Presentation | Location | Side | SM Grade | Size, mm | Eloquence* | Venous Drainage† | Flow-Related Aneurysm‡ | Time to Surgery, mo | Prior BAVM Treatment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 56 | H | Parieto-occipital | L | I | 27 | No | Superficial | No | 0.8 | No |
| 2 | M | 39 | H | Cerebellar | L | II | 36 | No | Superficial | Yes | 12.5 | No |
| 3 | M | 49 | H | Frontal | R | I | <10 | No | Superficial | No | 22.3 | Embolization§ |
| 4 | F | 26 | H | Choroid plexus | R | II | 21 | No | Deep | Yes | 0.7 | No |
| 5 | M | 66 | H | Temporal | L | II | <10 | Yes | Superficial | No | 14.9 | Embolization§ |
| 6 | M | 32 | E | Frontal | L | I | 25 | No | Superficial | No | … | No |
| 7 | M | 57 | H | Cerebellar | L | II | 34 | No | Superficial | Yes | 12.9 | No |
| 8 | M | 16 | H | Basal nuclei | R | V | 47 | Yes | Deep | Yes | 2.2 | Embolization§ |
| 9 | M | 43 | H | Frontal | R | I | 10 | No | Superficial | No | 1.8 | No |
| 10 | M | 68 | H | Frontal | L | I | 19 | No | Superficial | No | 8.7 | No |
| 11 | M | 19 | H | Frontal | R | I | 15 | No | Superficial | No | 10.2 | No |
| 12 | M | 44 | H | Corpus callosum | L | II | 28 | No | Deep | No | 0.5 | Surgery |

BAVM indicates brain arteriovenous malformation; E, epilepsy; F, female; H, hemorrhage; L, left; M, male; R, right; and SM Grade, Spetzler-Martin Grade.

*Any location involving the sensorimotor, language, or visual cortex; the (hypo)thalamus; the internal capsule; the brain stem; the cerebellar peduncles; or the deep cerebellar nuclei.

†Drainage was considered deep when draining in the internal cerebral, basal vein of Rosenthal or precentral cerebellar vein. In the posterior fossa, only veins emptying in either the straight or transverse sinuses were considered superficial.

‡Either on a feeding artery or within the nidus.

§Time interval between embolization and surgery was 5.8 (patient 3), 9.6 (patient 5), and 34.9 (patient 8) mo.

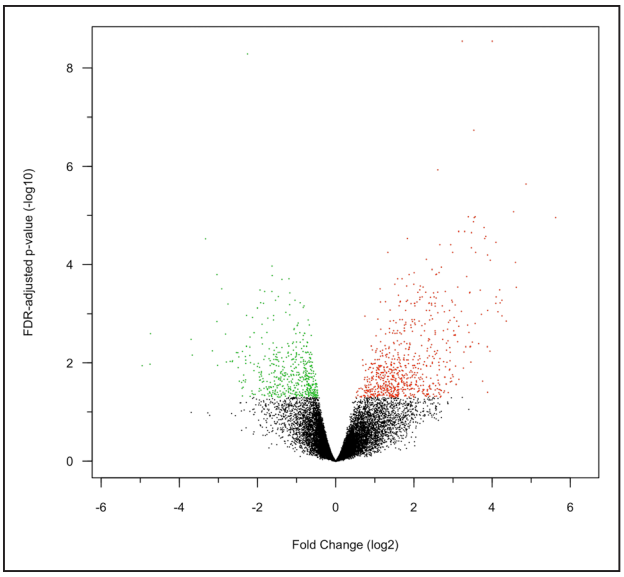


**Figure 1.** Volcano plot. Red indicates significantly (false discovery rate [FDR]-adjusted $P<0.05$) upregulated genes in brain arteriovenous malformations (BAVMs) whereas green indicates significantly downregulated genes as compared to intracranial control arteries. Black indicates genes not differentially expressed.

## Functional Pathway Analysis

Functional analysis of the 1234 differentially expressed genes showed the protein digestion and absorption pathway as significantly associated with BAVMs after FDR correction ($P=1.70\times10^{-2}$). This pathway mainly contained downregulated genes encoding for members of the collagen family (Table IV in the online-only Data Supplement). Two biologically related pathways, focal adhesion and extracellular matrix-receptor interaction, were no longer significantly associated after FDR correction ($P=9.15\times10^{-2}$ and $P=9.72\times10^{-2}$, respectively). These pathways consisted of downregulated genes encoding for extracellular matrix (ECM) constituents, including multiple integrins and collagens, heparin sulphate, fibronectin, and laminin. We identified 47 enriched GO terms (FDR-adjusted $P<0.05$) implicated in axon assembly, the actomyosin and microtubular cytoskeleton, motility and migration, ECM, vascular smooth muscle cells, voltage-gated (ion) channels, and (metal ion) transmembrane transport (Table V in the online-only Data Supplement).

## Genes and Signaling Pathways Previously Implicated in BAVMs

In BAVMs, we found downregulation of *SMAD4*, encoding for an intracellular effector downstream in the TGF-β signaling pathway, and regulating transcription. No other known causal genes of BAVMs (*KRAS*, *BRAF*, *ENG*, *ACVRL1*, *RASA1*, and *EPHB4*) showed differential expression. Analyzing the canonical TGF-β/BMP signaling pathway, we found additional genes mainly showing decreased expression in BAVMs, including genes encoding for latent binding proteins, transmembrane receptors, intracellular effectors, and transcription factors.

We found neither differential expression of VEGF nor its receptors nor of genes involved in Notch-1 signaling.

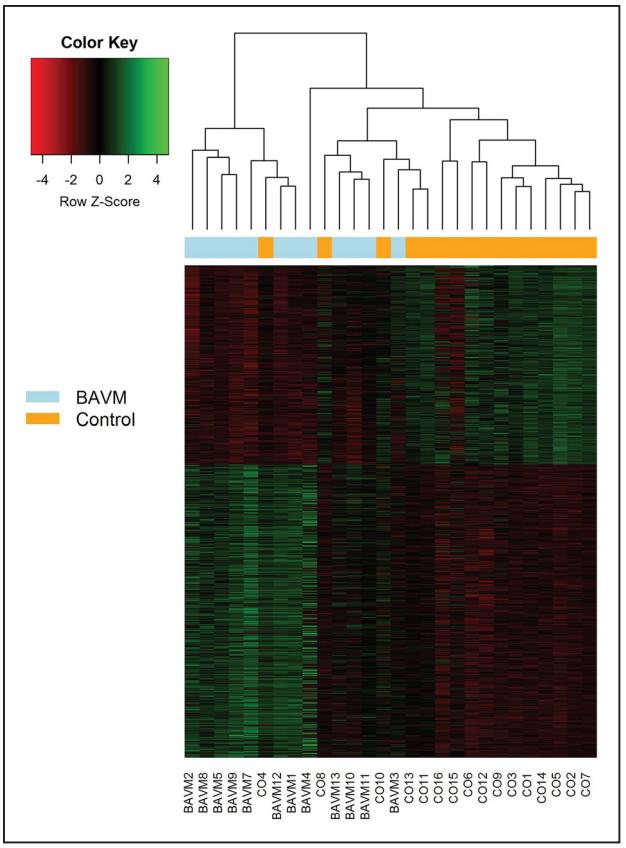


**Figure 2.** Heatmap. Unsupervised hierarchical clustering of the 1234 differentially expressed genes comparing 12 brain arteriovenous malformations (BAVMs) to 16 intracranial control arteries. The matrix consists of variance-stabilizing transformations of raw count data to be rid of the dependence of the variance on the range of means of logarithmic count data, allowing for proper clustering. The dendrogram shows the degree of similarity between pairs of samples based on the Euclidean distance. The Row Z-Score (the actual value of a gene per sample minus the mean value across all samples divided by the SD) represents an intensity-scaling factor that facilitates visualization of the row genes. Accordingly, red indicates decreased expression of a gene relative to the mean whereas green indicates increased expression. Overall, BAVMs show a distinct gene expression profile from those of intracranial control arteries.

By contrast, we found receptor tyrosine kinase (*TEK*, also known as *TIE-2*) downregulated in BAVMs. In addition, *ANGPT1* (*ANG1*), implicated in maintenance of EC quiescence, showed downregulation in BAVMs, whereas its competitive, proangiogenic antagonist on the binary TIE-2 receptor, *ANGPT2*, showed no upregulation in BAVMs.

## Discussion

Our study showed genes implicated in the cytoskeletal machinery and genes encoding for cytokines and secretory products of neutrophils and macrophages to be upregulated in BAVMs, indicating a role for inflammatory mediators in BAVMs. Furthermore, genes implicated in ECM composition, the binary angiopoietin-TIE system, and TFG-β signaling were downregulated. Pathways involved in (migratory) activity of the cytoskeleton, vascular wall structure, transmembrane transport, and endoplasmic reticulum are likely implicated in development, rupture or both of BAVMs.

**Table 2. Top 10 Differentially Expressed Genes in Brain Arteriovenous Malformations**

| Ensembl | Location | HGNC Symbol | Description | RNA-Sequencing Data | | | Droplet Digital PCR | |
|---|---|---|---|---|---|---|---|---|
| | | | | LR | logFC* | FDR-Adjusted *P* Value | logFC* | *P* Value† |
| ENSG00000124920 | 11q12.2 | *MYRF* | myelin regulatory factor | 53.92 | −4.00 | $2.86\times10^{-9}$ | | |
| ENSG00000004838 | 3p21.31 | *ZMYND10* | zinc finger, MYND-type containing 10 | 52.88 | −3.24 | $2.86\times10^{-9}$ | −3.73 | 0.002 |
| ENSG00000162733 | 1q23.3 | *DDR2* | discoidin domain receptor tyrosine kinase 2 | 50.93 | 2.26 | $5.16\times10^{-9}$ | 1.08 | <0.001 |
| ENSG00000197748 | 10q25.1 | *CFAP43* | cilia and flagella associated protein 43 | 43.36 | −3.53 | $1.84\times10^{-7}$ | −3.32 | 0.006 |
| ENSG00000267548 | NA | NA | | 39.29 | −2.61 | $1.18\times10^{-6}$ | | |
| ENSG00000180638 | 17p11.2 | *SLC47A2* | solute carrier family 47 (multidrug and toxin extrusion), member 2 | 37.63 | −4.87 | $2.30\times10^{-6}$ | −4.94 | <0.001 |
| ENSG00000133063 | 1q32.1 | *CHIT1* | chitinase 1 (chitotriosidase) | 34.80 | −4.55 | $8.42\times10^{-6}$ | −5.72 | 0.013 |
| ENSG00000197971 | 18q23 | *MBP* | myelin basic protein | 34.08 | −3.57 | $1.07\times10^{-5}$ | | |
| ENSG00000160401 | 9q34.11 | *C9orf117* | chromosome 9 open reading frame 117 | 33.85 | −3.39 | $1.07\times10^{-5}$ | | |
| ENSG00000012223 | 3p21.31 | *LTF* | lactotransferrin | 33.52 | −5.62 | $1.11\times10^{-5}$ | −5.68 | <0.001 |

FDR indicates false discovery rate; HGNC, HUGO Gene Nomenclature Committee; logFC, log-fold change; LR, likelihood ratio; NA, not available; and PCR, polymerase chain reaction.

*Negative fold changes indicate overexpression in brain arteriovenous malformations.

†Bonferroni corrected.



## Inflammation

Genes encoding for secretory products of neutrophils and macrophages showed upregulation in BAVMs with relatively high fold changes. Surgical specimens have shown prominent infiltration of these cells of innate immunity in the vascular wall of (un)ruptured BAVMs,[7] just as the presence of excessive amounts of MMPs (matrix metalloproteinases), such as myeloperoxidase and leukocyte-derived MMP-9.[7,19] In line with previous genome-wide expression studies on BAVM specimens,[8–10] we found many upregulated genes encoding for inflammatory cytokines, which result in recruitment of leucocytes. Consistent with studies that showed cell adhesion molecules on ECs using immunostaining, like E-selectin and intercellular cell adhesion molecules,[20] we found upregulation of *CERCAM* (cerebral EC adhesion molecule), implicated in transmigration of leucocytes across the blood-brain barrier (BBB). We found evidence for possible T-cell involvement, whose infiltration in BAVMs has been described only recently.[6] Subtyping of T-lymphocytes remains to be done. The same is true for exploring what role various immune cells fulfill in BAVMs, including their interplay. Chronic inflammation may be a contributing factor to progressive vascular wall weakening in BAVMs. Whether such a process is causal or rather a result of an inadequate BBB remains elusive, albeit proper cross-talk between components of the BBB has been found critical for maintaining CNS immune quiescence by minimizing the expression of cell adhesion molecules and EC trafficking.[21]

## Loss of Cerebrovascular Quiescence

The downregulation of *ANGPT1*, implicated in maintenance and survival of ECs, junctional sealing of ECs by tight junctions, and anti-inflammatory signaling, may be indicative of reduced release of *ANGPT1* by adjacent pericytes and astrocytes. Its downregulation, and that of its receptor (*TEK*), is consistent with previous studies.[9,18,22] These findings may imply loss of EC quiescence. Increased proliferation of ECs in BAVMs has been shown by Ki-67 and proliferating cellular nuclear antigen staining,[5,23] while cerebral vessels normally lack mitotic activity. BAVMs haboring activating *KRAS* mutations, which are unknown in our BAVM specimens, show in vitro enhanced migratory behavior of ECs due to constitutive MAPK-ERK (mitogen-activated protein kinase/extracellular signal-related kinase) signaling.[3] These observations may be indicative of vascular sprouting and are in harmony with our finding of multiple strongly upregulated genes in BAVMs implicated in cilia.[3,24]

In our study, neither *VEGFA* nor *ANGPT2* showed differential expression whereas they have previously been found overexpressed at the gene and the protein level.[8,17,18,25] The disparity in results may be due to tissue heterogeneity, post-translational modifications, or to the fact that in some studies most BAVMs had been treated by embolization in the days before surgery.[8,25,26]

We identified enriched terms implicated in the cytoskeleton network, endoplasmic reticulum, voltage-gated (ion) channels, and a variety of transmembrane transporters. Many of these seem compatible with observations of perinidal ECs enriched in filopodia, lysosomes, Weibel-Palade bodies, intracellular gaps at EC junctions and membrane protrusions, while abnormal pericytes showed numerous pinocytotic vesicles, filaments, and vacuoles.[24] These findings jointly point to

increased (intra)cellular activity in BAVMs. Because of the nonspecificity of many terms, no conclusions can be drawn from this study to which intracellular mechanisms they point, and to which cell types.

### Impaired Integrity of the BBB

The processes mentioned thus far may fit with but are not conclusive for a dysfunctional BBB. Of interest, impaired ECM secretion of Col4A1 and Col4A2, 2 major structural constituents of the basement membrane, resulting from rare coding variants in their respective genes, have been linked to intracerebral hemorrhage.[27] In BAVMs, nidal walls show chaotic rearrangement of collagen fibers[28]; both our pathway analyses further highlight their role in BAVMs. Basement membranes of so-called perinidal capillaries, an irregular network of highly fragile vessels immediately adjacent to the nidus,[29] have been found severely disrupted or lacking,[24] whereas nidi exhibit areas of thin and irregular layers of vascular smooth muscle cells at various stages of differentiation.[30] Damage to the vascular wall in BAVMs is a likely result of inflammatory-driven proteolytic degradation.[7,19] ECM assembly may also be hampered in BAVMs, leaving vessels immature and fragile, since we found genes implicated in ECM downregulated. Interestingly, this finding contradicts their previously reported upregulation in BAVMs.[8]

ECM results from TGF-β signaling in adherent mural cells (vascular smooth muscle cells and pericytes), in concert with Notch, whereas TGF-β signaling in ECs promotes mural cell recruitment, subsequent differentiation and EC-pericyte adhesion.[15] The common basement membrane, pivotal in maintenance of the BBB, inhibits mutual proliferation of ECs and mural cells. In our study, genes implicated in TGF-β/BMP signaling showed differential expression. In hereditary hemorrhagic telangiectasia, perturbed canonical TGF-β/BMP signaling in primarily ECs results in reduced mural coverage, leaving vessels fragile and prone to proliferation and migration.[15,31] In addition, defective processing of fluid shear stress in impaired *ACVRL1* signaling has been shown to result in shunt formation as well as in vessel fragility in the less well-perfused vessels.[32] Strongly reduced pericyte and astrocytic end-foot coverage of ECs in BAVMs may lead to immune cell infiltration, increased vessel diameter, and vascular permeability,[24,33] of which silent intralesional microhemorrhages[34] and perilesional albumin depositions[35] may be manifestations. Pericyte-deficient mice support such disease mechanisms, which show formation of chaotic vascular networks as well as regression of capillary branches, vessel fragility and tortuosity, and micro-aneurysms with increased proliferation of ECs.[34] These features, including prominent caliber dilatations are well-recognized in BAVMs,[29] and support the emerging concept of a dysfunctional BBB in BAVMs.

### Strengths and Limitations

Strengths of our study are use of next-generation sequencing and the inclusion of a relatively large set of BAVM and control samples, both challenging to acquire, as compared to previous genome-wide expression studies. In addition, the cortical arteries obtained during epilepsy surgery resemble intracranial arteries the most, and their vascular quiescence have previously been ascertained.[5] Our study has limitations. First, all but one patient with BAVM underwent surgical resection because of hemorrhage. Accordingly, we may have identified differentially expressed genes due to, at least in part, the hemorrhage rather than the molecular pathways of the BAVM itself. In most patients, however, considerable time had elapsed until surgical resection limiting the effect of the hemorrhagic event itself. Second, prior embolization was performed in 3 patients. Since these took place at least 5 months before surgery, the effect of ischemia on gene expression may be limited, but that of (altered) hemodynamic forces remains unknown. Third, our approach did not allow us to study gene expression in the distinct cell populations of the BAVM vasculature. Last, the still relatively limited number of available tissue samples limited us to account for additional clinical and radiological variables. This could make our results more susceptible to erroneous findings. We, therefore, emphasize the necessity for external validation.

### Conclusions, Future Research, and Therapeutic Implications

Our study exploring the transcriptomic landscape of BAVMs highlights a major role for inflammation, loss of cerebrovascular quiescence, and impaired integrity of the vascular wall in the pathophysiology of BAVM development, rupture, aftermath or a combination of these processes. External validation of our findings is needed. Processes appear closely tied with one another, but their precise interaction remains to be elucidated. Some of the identified genes and signaling pathways have been implicated in multiple cell types of BAVMs. Therefore, future functional studies may focus in more detail on the distinct cell populations and their interaction. Our study further lends support to potential receptivity of BAVMs to medical therapeutics, including those promoting vessel maturation, and anti-inflammatory and immune-modifying drugs.

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## Disclosures

None.

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