Differential Gene Expression in Human Cerebrovascular Malformations

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Abstract

OBJECTIVE—We sought to identify genes with differential expression in cerebral cavernous malformations (CCMs), arteriovenous malformations (AVMs), and control superficial temporal arteries (STAs) and to confirm differential expression of genes previously implicated in the pathobiology of these lesions.

METHODS—Total ribonucleic acid was isolated from four CCM, four AVM, and three STA surgical specimens and used to quantify lesion-specific messenger ribonucleic acid expression levels on human gene arrays. Data were analyzed with the use of two separate methodologies: gene discovery and confirmation analysis.

RESULTS—The gene discovery method identified 42 genes that were significantly up-regulated and 36 genes that were significantly down-regulated in CCMs as compared with AVMs and STAs ($P = 0.006$). Similarly, 48 genes were significantly up-regulated and 59 genes were significantly down-regulated in AVMs as compared with CCMs and STAs ($P = 0.006$). The confirmation analysis showed significant differential expression ($P < 0.05$) in 11 of 15 genes (angiogenesis factors, receptors, and structural proteins) that previously had been reported to be expressed differentially in CCMs and AVMs in immunohistochemical analysis.

CONCLUSION—We identify numerous genes that are differentially expressed in CCMs and AVMs and correlate expression with the immunohistochemistry of genes implicated in cerebrovascular malformations. In future efforts, we will aim to confirm candidate genes specifically related to the pathobiology of cerebrovascular malformations and determine their biological systems and mechanistic relevance.

Keywords

Cerebrovascular malformations (CVMs) are lesions with an abnormal vessel phenotype that predisposes patients to hemorrhagic strokes, seizures, focal neurological deficits, and other clinical manifestations (4,22). They include arteriovenous malformations (AVMs) and cerebral cavernous malformations (CCMs) and have distinct clinicopathological radiological profiles (14,30). The AVMs are tangled complexes of tortuous vessels representing fistulous connections between arteries and veins, and they lack an intervening capillary bed. They reveal preserved features of mature vessel wall phenotype altered by high flow and hemodynamic stress, including arterial, nidal, and venous aneurysms (5,6,28). The CCMs are characterized by caverns filled with blood or thrombus and are lined with a single layer of endothelial cells. These low-flow lesions are associated with brittle vasculature and repetitive oozing (5,6). The CCMs lack inter-endothelial cell tight junctions and mature vessel wall angio-architecture (8, 37).

Little is known about the mechanisms of genesis or the progression of these lesions. Several proteins are abnormally expressed in AVMs and CCMs. Our group and others have demonstrated by performing immunohistochemical analysis that vascular endothelial growth factor (VEGF) and the VEGF receptors VEGF-r1 (flt1) and VEGF-r2 (flk1) are overexpressed in both AVMs and CCMs compared with normal brain vessels (21,35). Fibronectin is expressed to a greater extent in CCMs than in AVMs, consistent with the proliferative immature vessel phenotype (21). Laminin and smoothelin, both of which reflect mature vessel wall phenotype, are underexpressed in CCMs as compared with AVMs (21,34). Distinct genes have been identified that predispose individuals to familial manifestations of these lesions, which is related to transforming growth factor-β receptor binding proteins (1,13,18,19) and the Krev Interaction Trapped 1 (krit1) signaling pathway (9,17,23,26,32) for AVMs and CCMs, respectively.

We hypothesize that different groups of genes are involved in the pathogenesis of AVMs and CCMs and that other genes are nonspecifically associated with both lesion types. In the experiments described in this article, we applied gene microarray analysis to correlate alterations in ribonucleic acid (RNA) transcription in AVMs and CCMs to the previously published abnormal protein expression in these lesions. In addition, we found numerous other

genes differentially expressed in one or both lesion types, which has not been reported previously.

PATIENTS AND METHODS

Patients and Lesions

A total of 11 specimens, including 8 CVMs (4 AVMs and 4 CCMs) and 3 normal vessels (superficial temporal arteries [STAs]), were obtained from 10 patients between November 2000 and December 2001. We obtained Institutional Review Board approval to perform our experiments. Each of the patients had unambiguous clinicopathological radiological characteristics of AVM or CCM without features of mixed lesions (14,30). The relevant clinical and lesion features of the cases are summarized in Table 1.

Preparation of RNA

At the time of surgical excision, a small fragment of the CCM, AVM, or STA specimen (0.04–5 g) was snap-frozen in liquid nitrogen. The RNA was isolated from these specimens with the use of a modification of the method of Chomczynski and Sacchi (7). Briefly, TRI Reagent (Molecular Research Center, Inc., Cincinnati, OH) was added at a volume of 1 ml/100 mg lesion, which immediately froze when it contacted the snap-frozen lesion specimen. Immediately after thawing, the lesion was homogenized three times for 20 seconds on ice with the use of a Polytron PT 1200 homogenizer (Kinematica AG, Littau, Switzerland), with 10-second rest intervals between pulses. The homogenate was incubated for 10 minutes at 58°C and was homogenized a second time as described above. After the addition of 0.1 vol 1-bromo-3-chloropropane, the homogenate was vortexed for 15 seconds and incubated on ice for 1 hour. After centrifugation at $6000 \times g$ for 30 minutes, the upper aqueous phase was transferred to a new tube. One-half volume of isopropanol was added. After mixing, the solution was incubated on ice for 1 hour. After centrifugation at $12,000 \times g$ for 30 minutes, the supernatant was removed. The RNA pellet was washed with 80% ethanol and resuspended in diethylpyrocarbonate-treated water. The RNA was affinity column-purified with the use of an RNeasy Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. The amount of RNA isolated is indicated in Table 1. First-strand complementary deoxyribonucleic acid (cDNA) was synthesized from the poly-A containing messenger RNA (mRNA) as indicated below.

Synthesis of Double-stranded cDNA

First-strand cDNA was synthesized from 1.4 to 5 μg total RNA with Superscript Choice System (Life Technologies, Inc., Gaithersburg, MD), using an oligo-deoxyribothymidine primer containing a T7 RNA polymerase promoter (Genset Corp., Kents Store, VA). After second-strand synthesis, double-stranded cDNA was purified with phenol/chloroform extraction and ethanol precipitation.

Synthesis of Biotin-labeled complementary RNA and Hybridization

Biotin-labeled complementary RNA (cRNA) was synthesized by performing in vitro transcription with the use of the Bioarray High Yield RNA Transcript Labeling Kit (ENZO, Farmingdale, NY). The cRNA was purified by using an RNeasy Mini Kit (Qiagen, Inc.) and fragmented to between 50 and 200 bases in length by incubation at 94°C for 35 minutes in a fragmentation buffer containing 40 mmol/L Tris-acetate, pH 8.1, 100 mmol/L potassium acetate, and 30 mmol/L magnesium acetate. The cRNA was added at a concentration of 0.05 μg/μl to a hybridization solution containing 100 mmol/L morpholinoethanesulfonic acid, 1 mol/L sodium cation, 20 mmol/L ethylenediamine tetra-acetic acid, and 0.01% Tween 20.

Hybridization, Washing, and Staining of the Test Chip and the GeneChip

The sample of 200 μl was hybridized to the Affymetrix GeneChip test chip arrays (Affymetrix, Inc., Santa Clara, CA) by incubation at 45°C for 16 hours with the use of a GeneChip Hybridization Oven 640 (Affymetrix, Inc.). The arrays were washed, and then they were stained with streptavidin-phycoerythrin with the use of a GeneChip Fluidics Station Number 400 (Affymetrix, Inc.). Arrays were read at a resolution of 3 μm with an HP Gene Array Scanner (Affymetrix, Inc.). The sample was considered to have passed the test chip if the ratios of the relative expression of the 3′ end over the 5′ end were less than 3 for both the β-actin and the glyceraldehyde phosphate dehydrogenase genes that were present on the test chip. If the test chip passed, the sample was hybridized to the Affymetrix GeneChip Hu95a arrays containing 12,625 genes as described above for the test chip. Scanned chips produced an image data (*.dat) file.

Data Preprocessing

Detailed protocols for data analysis of Affymetrix microarrays were described previously (15,16,24). Expression of transcripts from each of 12,625 genes was assessed in each of the specimens and analyzed with the use of Microarray Suite version 5 software (Affymetrix, Inc.) (Fig. 1). The data from the 16 to 22 perfectly matched probes for each gene contained in the *.dat file were averaged to derive the data for the cell intensity (*.cel) file. A scaling factor was applied to the normalized data from the *.cel files to bring the average intensity for all probes on the array to 500, forming the analysis output (*.chp) file with the use of Microarray Suite version 5 software. This procedure allowed any sample to be normalized for comparison with other samples.

Data Analysis for Unbiased Gene Discovery

The data contained in the *.chp file were loaded into the Matlab version 6.00 release 12 software (The MathWorks, Natick, MA) for statistical analysis. The Affymetrix control probes and the genes, which were called Absent by Microarray Suite across all samples, were removed. A median filter was applied to the remaining genes to remove genes that did not vary statistically across the samples. This filter computed a statistic W for each gene, where $W = (n - 1) \times$ (variance) $\div$ median (variance), n is the number of samples, and median (variance) is the median value of all the variances calculated for each gene.

Assuming that most genes do not vary significantly across all samples, the genes in general demonstrate low variance across samples. Therefore, the W statistic uses the variance to determine the genes that vary significantly. A greater W value means greater variation. Assuming that the variances are random and that the noise is distributed normally, the W statistic is approximately χ^2 with $n - 1$ df. Hence, a P value for each gene can be determined, capturing the probability that null hypothesis of no significant variation is rejected. A multiple comparison correction was conducted on the basis of the false discovery rate (FDR), which controls the expected fraction of the null hypotheses rejected mistakenly (10), where FDR = (number of mistaken H_0 rejections) $\div$ (total number of H_0 rejections). An FDR of 10% was used with the P values for the χ^2 statistic W.

We separately assessed the groups of genes in which the intensities of gene transcription from the CCM or AVM samples were higher or lower than the intensities of the other lesion type or the STA samples and that were determined to be significant according to the Kruskal-Wallis rank-sum test. The groups were combined to yield the statistically significant genes for each combination. We catalogued these genes according to potential function whenever possible with the use of GeneSpring version 4.1 software (Silicon Genetics, Redwood City, CA).

Fold change was calculated by using the formula $[(I - R) \div \min(I, R)] + A,$ where I is the median of all values for gene expression in the specimens of interest, R is the median of all values for gene expression in the remaining specimens with which the specimens of interest were compared, $\min(I, R)$ is the minimum of I and $R,$ and $A = 1$ if I is greater than or equal to R or $A = -1$ if I is less than R.

Heritable forms of CCM mapped to 7p13-15 and 3q25.2-27 result from genes that are yet to be identified. The GeneSpring software program was used to identify genes on the Affymetrix chip that mapped to these physical locations on the human chromosomes. These mapped genes were examined to determine whether they were differentially expressed between groups of specimens.

Data Analysis for the Confirmation of Differential Expression of Known Genes

The *.chp file, which contained the scaled data, was converted to an Excel file. GeneSpring version 4.1 software (Silicon Genetics, Redwood City, CA) was used to find the genes of interest. We initially considered the transcription levels of genes known to affect vasculogenesis, angiogenesis, or vascular wall maturity; genes related to familial AVM and CCM disease; and genes related to proteins previously shown by our group and others to be expressed abnormally in these lesions.

The GraphPad InStat version 2.04a software program (GraphPad Software, San Diego, CA) was used on a DOS platform. Initially, the F distribution was used to determine whether the difference between the two standard deviations from the relative expression of a gene from a group of similar lesions was significant. If this difference was not significant, the unpaired t test was used. If the difference was significant ($P < 0.05$), either the nonparametric Mann-Whitney U test or the Welch approximate t test was used. One-tailed probabilities were used if there was an expectation regarding the direction of the differences of the means from previous investigations. Otherwise, the two-tailed probabilities were used. $P < 0.05$ was considered significant. The results of this Instat analysis were compared with the statistical analysis for these genes with the use of the unbiased gene discovery approach.

RESULTS

Total RNA obtained from four AVMs, four CCMs, and three STAs were studied. An additional CCM specimen failed to yield high-quality RNA. Data were analyzed with the use of the gene discovery approach and the confirmation of known gene methodologies (Fig. 1).

Gene Discovery in Cerebrovascular Malformations

After control and absent genes were removed from the scaled data, a median filter was applied to discard genes that did not vary significantly across experiments. After an FDR of 10% was used with the P values for the χ^2 statistic $W,$ 3178 genes were selected from the original 12,625 genes. Nonparametric Kruskal-Wallis rank-sum comparisons were performed for AVMs versus CCMs, AVMs versus STAs, and CCMs versus STAs. For the AVM versus CCM comparison, 223 genes met the rank-sum criteria for significance at 0.48 FDR with $P = 0.0286$ for each gene. For the AVM versus STA comparison, 1280 genes met the rank-sum criteria for significance at 0.17 FDR with $P = 0.0571$. For the CCM versus STA comparison, 1533 genes met the rank-sum criteria for significance at 0.14 FDR with $P = 0.0571$. The intersections of the AVM versus CCM and CCM versus STA comparisons resulted in genes that were differentially expressed in the CCMs compared with the other two groups ($P = 0.006$). The intersections of the AVM versus CCM and the AVM versus STA comparisons resulted in genes that were differentially expressed in the AVMs compared with the other two groups ($P = 0.006$).

Table 2 shows the 42 genes up-regulated in CCMs compared with AVMs and STAs. These include genes for 2 binding proteins, 7 enzymes, 13 immunoglobulins, 3 membrane proteins, 11 unclassified known (miscellaneous) genes, and 6 genes unknown to date. Fold change reflects the median relative gene expression in CCMs compared with median AVM and STA levels.

Table 3 shows the 36 genes down-regulated in CCMs compared with AVMs and STAs. This group includes many genes expressed in the smooth muscle cells of arteries, such as those that code for 6 myosin and 9 other cellular component proteins, including smoothelin, actin, and desmin. In addition, this group contains genes for two apoptosis-related proteins, five enzymes, two heat shock proteins, nine unclassified proteins, and three genes unknown to date. Fold change reflects the median relative combined gene expression in AVMs and STAs compared with median CCM levels. Figure 2 shows the scan of the microarray cells containing the 16 perfectly matched oligonucleotide probes for the smooth muscle myosin heavy chain (D10667) hybridized to biotin-labeled cRNA from representative vascular specimens.

Table 4 indicates the 48 genes up-regulated in AVMs when compared with CCMs and STAs. These include genes coding for 8 enzymes, 5 nuclear proteins, 11 receptors, 4 ubiquitin-related proteins, 16 unclassified genes, and 4 genes unknown to date. Fold change reflects the median relative gene expression in AVMs compared with median CCM and STA levels.

Table 5 shows the 59 genes down-regulated in AVMs compared with CCMs and STAs. These include genes for 2 apoptosis-related proteins, 5 binding proteins, 4 cell adhesion proteins, 8 enzymes, 4 oncology-related proteins, 2 receptor ligands, 3 transcriptional regulators, 2 translation initiation factors, 2 transporters, 14 unclassified genes, and 13 genes unknown to date. Fold change reflects median relative combined gene expression in CCMs and STAs compared with median AVM levels. In addition, 558 genes (not shown) were up-regulated in STAs compared with AVMs and CCMs ($P = 0.012$), and 310 genes were down-regulated in STAs compared with AVMs and CCMs ($P = 0.012$). The functional classification of these genes is beyond the scope of this article.

CCM is an oligogenic trait with genetic contributions from three loci. Mutations in the *CCM1* gene cause CCMs. The other two genes map to the 7p13-15 region (CCM2) and the 3q25.2-27 region (CCM3) (9). The CCM2 and CCM3 genes have not been identified. In our experiment, 2 of 38 genes on the gene array that mapped to the 7p13-15 region were differentially expressed. Gene expression for *DFNA5* (AF073308), which maps to the 7p15 region within CCM2, is up-regulated in CCMs compared with AVMs and STAs (3.7-fold, *P* = 0.006; Table 2). Gene expression for *TAX1BP1* (U33821), which also maps to 7p15, is down-regulated in AVMs and CCMs compared with STAs (1.4 fold, *P* = 0.012). One of 32 genes on the gene array that mapped to 3q25.2-27 was differentially expressed. Gene expression of *KCNAB1* (L39833 and X83127), which to mapped to the 3q26.1 region within CCM3, is down-regulated in AVMs and CCMs compared with STAs (20.3- and 22.7-fold, *P* = 0.012).

Confirmation of Differential Expression of Known Genes in Cerebrovascular Malformations

In previous investigations, several proteins were found to play a role in the molecular structure or function of AVMs and CCMs. These include krit1, endoglin (CD105), activin receptor-like kinase (alk1), VEGF, flt1, flk1, receptor tyrosine kinases tie1 and tie2, fibronectin, laminin, smoothelin, and inter-endothelial cell tight junction protein. The relative mRNA expression derived from microarrays for these proteins is shown in Figure 3.

The Krit1 *CCM1* (accession U90268) gene is expressed less in AVMs than in CCMs (*P* = 0.028) or in STAs (*P* = 0.008). There is a trend for *CCM1* to be expressed less in CCMs than in STAs (*P* = 0.17). Expression of the endoglin *ENG* (X72012) gene is higher in CCMs than

in STAs ($P = 0.038$). There is only a slight trend for the *ACVRL1* (Z22533) gene to be expressed more in AVMs than in CCMs ($P = 0.48$) or in STAs ($P = 0.49$). There is a trend for the *VEGF* (M63978) gene to show greater expression in AVMs ($P = 0.057$) and CCMs ($P = 0.057$) than in STAs. The flt1 *ZYX* (X51602) gene is expressed more in AVMs than in STAs ($P = 0.038$). The kinase insert domain receptor flk1 *KDR* (AF035121) gene is expressed less in CCMs than in STAs ($P = 0.026$). Less expression of angiopoietin-1 *ANGPT1* (U83508) gene is observed in both AVMs ($P = 0.031$) and CCMs ($P = 0.038$) than in STAs. The *TIE* (X60957) gene is expressed less in AVMs than in STAs ($P = 0.045$), and there is a trend for less expression of the *TIE* gene in CCMs than in STAs ($P = 0.089$). There is less expression of the *TEK* (L06139) gene in AVMs than in CCMs ($P = 0.060$) or in STAs ($P = 0.020$). Although there is less expression of the $\alpha3$ chain of the laminin *LAMA3* (L34155) gene in AVMs ($P = 0.021$) and CCMs ($P < 0.0001$) than in STAs, there is only a trend for less expression in CCMs than in AVMs ($P = 0.10$). The smoothelin *SMTN* (AI888563) gene is expressed less in CCMs than in AVMs ($P = 0.014$) or in STAs ($P = 0.006$), and it is expressed less in AVMs than in STAs ($P = 0.008$). There is a slight trend for greater expression of the fibronectin FN (HG3044-HT3742) gene in CCMs than in AVMs ($P = 0.12$).

The NPXY motif of the integrin cytoplasmic domain-associated protein (ICAP)-1α protein interacts with both krit1 and integrin $\beta1$ (38). There is a trend for less expression of *ICAP-1A* (AF0121023) gene in CCMs than in STAs ($P = 0.125$) and AVMs ($P = 0.481$). There is less expression of platelet endothelial cell adhesion molecule (CD31) *PECAM1* (L34657) gene in AVMs ($P = 0.037$) and CCMs ($P = 0.046$) than in STAs. Less expression is observed for the gap junction protein *GJA4* connexin 37 (M96789) gene in AVMs ($P = 0.0008$) and CCMs ($P < 0.0001$) than in STAs, and less expression is seen in CCMs than in AVMs ($P = 0.014$).

When the rank-sum unbiased gene discovery approach is applied to 15 select genes of interest, differential expression for 9 genes is found to be significant. When STAs are compared with AVMs, gene expression for *CCM1, TIE, TEK, LAMA3, SMTN, PECAM1, GJA4,* and *ANGPT1* is significantly different. When STAs are compared with CCMs, gene expression for *KDR, LAMA3, SMTN, GJA4,* and *ANGPT1* is significantly different. When AVMs are compared with CCMs, the mRNA expression for *SMTN* and *GJA4* is significantly different.

The expression for the *ENG* and *ZYX* genes is significantly different when the confirmation approach is used but not when the gene discovery approach is used, owing to differences in analytical methods (Fig. 1). In the confirmation approach, mRNA expression is significantly down-regulated for *CCM1* when AVMs are compared with both CCMs and STAs, and for *PECAM1* when both AVMs and CCMs are compared with STAs. The rank-sum gene expression approach shows that mRNA expression of these genes is significantly different only when AVMs are compared with STAs.

DISCUSSION

The pathobiology of CVMs is clearly related to abnormal vascular assembly or maintenance. AVMs exhibit mature vessel wall elements with direct communication between arteries and veins and a high-flow profile predisposing to vascular recruitment, arterialization of venous structures, and gliosis of intervening and adjacent brain tissue. They are prone to apoplectic hemorrhage by rupture of nidal vessels or associated aneurysms or by venous outflow obstruction (5,6,28). By contrast, CCMs seem to grow by a process of proliferation of vascular caverns in the setting of repetitive lesional hemorrhages and exhibit brittle vascular morphology devoid of mature vessel wall elements (5,6,37). CCMs do not exhibit the high-flow features of AVMs and are less commonly associated with apoplectic hemorrhage (5,6).

Patients with CCMs often present with epilepsy or progressive focal neurological deficits as a result of repetitive lesional bleeding (4,22).

The molecular milieu of CVMs likely reflects factors that affect lesion genesis and secondary pathophysiological alterations such as associated inflammation, ischemia, hemorrhage, and gliosis. Some of these mechanisms may be similar in all CVMs, but others may be specific to individual lesion types or to particular lesion behavior (e.g., hemorrhage, epilepsy). The molecular structure of CVMs has been explored by performing immunohistochemistry (21, 34,35), in situ hybridization (33), and other techniques (8,37). Previous studies conducted in our laboratory and by others have demonstrated the underexpression of proteins characterizing the mature vessel wall phenotype in CCMs as compared with AVMs (21,34). Angiogenesis factors, including VEGF, are overexpressed in both AVMs and CCMs as compared with control brain vessels, but there is apparent differential expression of VEGF and angiopoietin receptors in the two lesion types (21,35). These observations have largely been qualitative and have focused on a limited number of candidate molecules. More complex gene interactions are likely involved in the genesis and subsequent biological behavior of the different lesions. Genes that predispose individuals to common familial forms of CCMs and rare familial AVMs were recently identified (1,9,13,15,17–19,23,26,32). The role of these genes and the related pathways in the pathobiology of CVMs is not known.

The differential expression of scores of genes may be examined simultaneously with the use of gene array techniques to compare mRNA levels in tissue from various sources by performing bioinformatic analysis. These approaches have not been applied previously in the study of CVMs. We sought to compare gene expression in AVMs, CVMs, and STAs for differential gene discovery and to confirm the role of specific genes in the pathobiology of these lesions.

Differential Gene Discovery in AVMs and CCMs

We used a gene discovery approach to identify groups of genes differentially expressed in CVMs. We categorized genes over- or underexpressed in CCMs and AVMs compared with the other lesion types and STAs. We applied a threshold of differential expression in each AVM or CCM specimen as compared with the other lesion type and the control STA specimen as well as a second threshold of statistical significance measured by rank-sum correlation. This allowed the identification of genes significantly over- or underexpressed in a specific lesion type (AVM or CCM) as compared with other CVMs and a control vessel, as well as genes differentially expressed in both types of CVMs compared with STAs.

There were 42 genes with significantly greater expression in each CCM specimen than in every AVM and STA specimen. This group included the overexpression of 13 immunoglobulin genes in CCMs, suggesting the possible role of a CCM-specific antigen that would stimulate the recruitment of B-lymphocytes with the production of antibodies. Of particular note is a 43-fold increase in mRNA from CCMs for an allele of the *DQW1-β* gene from major histocompatibility complex Class II *HLA-DR2-DW12,* also reflecting an apparently unique immune response in CCMs. In addition, a fourfold increase in mRNA was noted in CCMs of a gene coding for selenoprotein P, a plasma protein associated with vascular endothelium and a prime target for peroxynitrite toxicity (29).

Likewise, 36 genes were differentially down-regulated in CCMs compared with AVMs and STAs. As expected, genes coding for components of smooth muscle cells were underexpressed in CCMs, including myosin, smoothelin, filamen A endothelial actin binding protein, basic smooth muscle calponin 1, α-actin, desmin, tropomodulin tropomyosin binding protein, and transgelin 22DA smooth muscle protein. Also important with regard to the characteristics of the CCM are the 4.6-fold decrease in mRNA expression of microfibril-associated glycoprotein 4 and the 3.1-fold decrease in endothelium-specific connexin 37 (*GJA4*) gap junction protein.

The AVMs contained 48 genes that were differentially up-regulated compared with CCMs and STAs. Most notable was the 17-fold increased expression in AVMs of the gene coding for the interleukin-8 receptor type B, splice variant *IL8RB1*. Also, AVMs showed a 10-fold increased mRNA expression for ST2 interleukin-1 receptor-like 1 protein and an 8-fold increased mRNA expression of maltose-glucoamylase enzyme.

The AVMs contained 59 genes that were differentially down-regulated compared with CCMs and STAs. Most of these genes were down-regulated less than threefold. Two exceptions were the insulin-like growth factor 1 mRNA, which down-regulated by sevenfold, and the pre-B cell stimulating factor homolog (*SDF1B*) chemokine mRNA, which down-regulated by fourfold.

Heritable forms of CCM are found in 15 to 50% of individuals with CCMs (17) and map to the CCM1, CCM2, and CCM3 loci. Although the *CCM1* gene is the CCM1 locus, the genes for CCM2 and CCM3 have not yet been identified. Mapping within the CCM2 locus, the gene *DFNA5*, which is associated with nonsyndromic hearing impairment and is inversely correlated with estrogen receptor expression in breast carcinoma (36), is up-regulated for mRNA expression in CCMs compared with AVMs and STAs (Table 2). Also mapping within the CCM2 locus, the gene *TAX1BP1*, which codes for the TAX1-binding protein 1, a tumor necrosis factor receptor-associated factor 6-interacting protein involved in interleukin-1 signaling (25), is down-regulated for mRNA expression in AVMs and CCMs compared with STAs. Mapping to 3q26.1 within the CCM3 locus, the gene *KCNAB1*, which codes for the β subunit member 1 of voltage-gated shaker-related potassium channel (20), is also down-regulated for mRNA expression in AVMs and CCMs compared with STAs. These results make these candidate genes of particular interest.

Differential Expression of Selected Genes with Potential Pathobiological Significance

Fifteen genes were present on the arrays that were suggested previously to have molecular structural or functional roles in the pathobiology of AVMs and CCMs. Mutations resulting in the truncation of the krit1 protein cause CCM1, one of three familial genotypes of CCM. Although the mRNA expression of *CCM1* is significantly down-regulated in AVMs compared with both CCMs and STAs, there is only a trend for less expression in CCMs than in STAs ($P = 0.17$). Krit1 competes with integrin β1 for the NPXY motif of ICAP-1α (38). There is a trend for less mRNA expression of *ICAP-1A* in CCMs than in STAs. Of interest is the down-regulation by 1.5-fold of integrin β5 subunit *ITGB5*, and by 1.9-fold of integrin α6 *ITGA6* mRNA expression, in AVMs (Table 5), which suggests a role in both CCMs and AVMs. Integrin binds to the sequence arginine-glycine-aspartate of several adhesive proteins in extracellular matrices, including laminin and fibronec-tin (31).

Previous investigations showed the underexpression of laminin and smoothelin proteins in CCMs as compared with AVMs. Both of these proteins reflect a mature vessel wall phenotype (21,34). In our study, gene expression for both *LAMA3* and *SMTN* mRNA was down-regulated in both AVMs and CCMs as compared with STAs. There is a trend for lower mRNA expression of *LAMA3* in CCMs than in AVMs, at a probability approaching significance ($P = 0.01$). Also, gene expression for *SMTN* mRNA is down-regulated in CCMs as compared with AVMs.

Fibronectin proteins are expressed more in CCMs than in AVMs, which is consistent with immature vessel phenotype. In our study, there was a slight trend for more mRNA expression of *FN* in CCMs than in AVMs, at a probability approaching significance ($P = 0.12$).

Endoglin and alk1 are proteins involved in familial AVM, hereditary hemorrhagic telangiectasia Types 1 and 2 (HHT1 and HHT2). Gene expression for *ENG* mRNA is up-regulated in both AVMs and CCMs compared with STAs. There is a slight but insignificant

up-regulation in the mRNA expression of *ACVRL1* in AVMs compared with both CCMs and STAs. Previous studies showed the overexpression of endoglin proteins in patients with sporadic and familial AVMs (27). The significance of the overexpression of genes responsible for familial CVMs in the lesions themselves remains unclear and merits further study.

The proteins VEGF and its receptors flt1 and flk1 were previously shown to be overexpressed in both AVMs and CCMs compared with normal blood vessels (18,32). In our study, mRNA expression of *VEGF* was up-regulated in both AVMs and CCMs compared with STAs, at a probability approaching significance ($P = 0.057$). More mRNA expression was shown for *ZYX* in AVMs than in STAs. However, mRNA expression of *KDR* is down-regulated in CCMs compared with STAs.

Angiopoietin-1 and its receptors tie1 and tie2 are involved in angiogenesis. Gene expression of angiopoietin-1 *ANGPT1* mRNA is down-regulated in both AVMs and CCMs compared with STAs. Gene expression of mRNA for both *TIE* and *TEK* is down-regulated in AVMs compared with STAs.

Gene expression of *PECAM1* is lower in both AVMs and CCMs than in STAs. Decreased mRNA expression is shown for the endothelium-specific gap junction protein *GJA4* connexin 37 in both AVMs and CCMs compared with STAs, and less expression is observed in CCMs than in AVMs, which are consistent with previous reports of tight junctions lacking in CCMs (8,37).

Methodological Issues and Limitations

Although microarrays have introduced a powerful methodology for identifying scores of genes associated with a particular disease, they have limitations that may lead to discrepancies. One recent editorial proposed that results obtained by performing microarray experiments be validated through 1) reproducibility and control for run-to-run variations in gene expression, 2) statistical correction for repeated and multiple comparisons, 3) attention to tissue homogeneity, and 4) confirmation of results with the use of nonarray techniques (12).

For unbiased gene discovery, the Bonferroni correction for repeated and multiple comparisons is too stringent and would be expected to yield almost no results. Instead, the multiple comparison correction used in our experiment was based on the most stringent threshold for FDR in the Kruskal-Wallis rank-sum analysis of the genes that varied significantly. We hence included all genes that satisfied this threshold. A fold change cutoff would have biased the comparisons, so we reported the fold change for any differential expression that was statistically significant. The Kruskal-Wallis test is non-parametric, hence a mean and standard deviation was not included in the gene discovery results. Because most of the genes used in the confirmational analysis were chosen on the basis of a priori knowledge gained by performing immunohistochemistry, a more traditional statistical analysis was used in comparing the intensity of expression of known genes.

There is an FDR inherent in analyzing a large amount of data, the specificity of the oligonucleotide, and the variation in stringency conditions used in the oligonucleotide array. Genes that code for proteins with multiple isoforms and splice variants can complicate the microarray analysis. A potential example of such discrepancy was the expression of *KDR,* in which mRNA expression was lower in CCMs than in normal STAs, whereas previous immunohistochemical studies had shown greater expression in the lesions than in normal cerebral vessels (35). Another possible cause of this discrepancy is the different control tissue used (brain vessels versus STA) in the two studies.

Another major challenge with the use of emerging microarray technology is the problem of tissue heterogeneity. Many genes are cell-specific (i.e., VEGF receptors), and most lesions and control tissue are composed of multiple cell types. The pooling of mRNA or protein from lesional tissue inherently reflects and is affected by such heterogeneity. There might be relevant differential expression of genes in endothelial, smooth muscle, and other cells. Gene expression reflects the differential cell composition of lesions and/or the differential expression of genes in a particular cell type. For example, it is not surprising that a lesion such as a CCM, which lacks smooth muscle cells, is likely to exhibit underexpression of smooth muscle-related genes. Conversely, two cases of robust differential overexpression of immune system genes in CCMs, as compared with AVM and control tissue, force us to consider potential inflammatory mechanisms that are unique to these lesions. Differential gene expression in complex systems should be confirmed and further characterized in complementary experiments focused on the specific gene and the relevant cell line. Such experiments may include immunohistochemistry (34), in situ hybridization (33), or other techniques.

The selection of relevant controls is another potential source of bias. In this study, different lesion types were used for control comparisons of gene expression (AVM versus CCM) as well as normal scalp artery (lesion versus STA). Dissected cerebral vessels from surgical or autopsic tissue might provide additional meaningful comparisons in future studies, although they introduce new biases related to vessel-associated diseases and tissue harvesting. Cultured cell lines introduce other biases related to changes in phenotype and gene expression in vitro (2). Laser-dissected cell layers (i.e., endothelial layers) from pathological tissue were recently used to examine differential gene expression in a particular cell type (3). Such studies are needed to clarify the roles of specific genes and signaling pathways (as in the differential expression of *KDR* in isolated endothelial cells from lesions and controls and assayed levels of protein extracted from these cells).

Relevance and Future Research

In our study, 72 genes were differentially expressed in CCMs compared with AVMs. Many of these genes may be specifically involved in the cause or associated biological behavior of CCMs, and others may reflect associated thrombosis, vascular leakage, or epileptogenicity that are manifested in these lesions. Many of the 107 genes differentially expressed in AVMs compared with CCMs and STAs may be involved specifically in the AVM disease, and others may reflect features of arteriovenous shunting or secondary sequelae. Some of the 868 genes differentially expressed in both AVMs and CCMs compared with STAs may be related to vascular dysmorphism or its sequelae in either lesion type. These genes need to be cataloged according to their functional significance. Future studies should correlate differential gene expression with particular phenotypic, genotypic, or clinical features of the disease, such as CCMs with or without associated venous anomalies, lesions of different genotypic substrate, and lesions associated with hemorrhage or epilepsy. Considerable clinical heterogeneity exists even within familial forms of CCMs and AVMs. The differentially expressed genes described in our experiments are candidates for genetic modifiers of CCMs and AVMs.

It is known that for some genes, alterations in gene transcription may not necessarily affect the amount of protein present in the specimens (39). An analysis of differential proteomics with the use of mass spectrometry (11) should be conducted in AVMs and CCMs to confirm whether differential gene expression indeed reflects differences in the quantity of proteins produced by these genes and whether differential expression in the lesions reflects a complex, heterogeneous, and multicellular milieu. Candidate genes and related pathways should be studied further in homogeneous single-cell systems (cultured lines or laser-dissected cells) to confirm specific hypotheses regarding their role in CVM pathobiology.

Acknowledgments

References

1. Abdalla SA, Pece-Barbara N, Vera S, Tapia E, Paez E, Bernabeu C, Letarte M. Analysis of ALK-1 and endoglin in newborns from families with hereditary hemorrhagic telangiectasia type 2. Hum Mol Genet 2000;9:1227–1237. [PubMed: 10767348]

2. Baev NI, Awad IA. Endothelial cell culture from human cerebral cavernous malformations. Stroke 1998;29:2426–2434. [PubMed: 9804658]

3. Ball HJ, McParland B, Driussi C, Hunt NH. Isolating vessels from the mouse brain for gene expression analysis using laser capture microdissection. Brain Res Brain Res Protoc 2002;9:206–213. [PubMed: 12113780]

4. Brown, RD, Jr. Epidemiology and natural history of vascular malformations of the central nervous system. In: Jafar, JJ.; Awad, IA.; Rosenwasser, RH., editors. Vascular Malformations of the Central Nervous System. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 129-148.

5. Bruner, JM.; Tien, RD.; McLendon, RE. Tumors of vascular origin. In: Bigner, DD.; McLendon, RE.; Bruner, JM., editors. Russell and Rubinstein's Pathology of Tumors of the Nervous System. Vol. 6. Vol. 2. London: Arnold; 1998. p. 239-293.

6. Challa VR, Moody DM, Brown WR. Vascular malformations of the central nervous system. J Neuropathol Exp Neurol 1995;54:609–621. [PubMed: 7666048]

7. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–159. [PubMed: 2440339]

8. Clatterbuck RE, Eberhart CG, Crain BJ, Rigamonti D. Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations. J Neurol Neurosurg Psychiatry 2001;71:188–192. [PubMed: 11459890]

9. Craig HD, Gunel M, Cepeda O, Johnson EW, Ptacek L, Steinberg GK, Ogilvy CS, Berg MJ, Crawford SC, Scott RM, Steichen-Gersdorf E, Sabroe R, Kennedy CT, Mettler G, Beis MJ, Fryer A, Awad IA, Lifton RP. Multilocus linkage identifies two new loci for a Mendelian form of stroke, cerebral cavernous malformation, at 7p15-13 and 3q25.2-27. Hum Mol Genet 1998;7:1851–1858. [PubMed: 9811928]

10. Curran-Everett D. Multiple comparisons: philosophies and illustrations. Am J Physiol Regul Integr Comp Physiol 2000;279:R1–R8. [PubMed: 10896857]

11. Fessler MB, Malcolm KC, Duncan MW, Worthen GS. A genomic and proteomic analysis of activation of the human neutrophil by lipopolysac-charide and its mediation by p38 mitogen-activated protein kinase. J Biol Chem 2002;277:31291–31302. [PubMed: 11943771]

12. Firestein GS, Pisetsky DS. DNA microarrays: Boundless technology or bound by technology? Guidelines for studies using microarray technology. Arthritis Rheum 2002;46:859–861. [PubMed: 11953960]

13. Gallione CJ, Scheessele EA, Reinhardt D, Duits AJ, Berg JN, Westermann CJ, Marchuk DA. Two common endoglin mutations in families with hereditary hemorrhagic telangiectasia in the Netherlands Antilles: Evidence for a founder effect. Hum Genet 2000;107:40–44. [PubMed: 10982033]

14. Garcia, JH.; Anderson, ML. Circulatory disorders and their effects on the brain. In: Davis, RL.; Robertson, DM., editors. Textbook of Neuropathology. Vol. 2. Baltimore: Williams & Wilkins; 1991. p. 621-718.

15. Geraci MW, Moore M, Gesell T, Yeager ME, Alger L, Golpon H, Gao B, Loyd JE, Tuder RM, Voelkel NF. Gene expression patterns in the lungs of patients with primary pulmonary hypertension: A gene microarray analysis. Circ Res 2001;88:555–562. [PubMed: 11282888]

16. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, Lander ES. Molecular classification of cancer: Class discovery

and class prediction by gene expression monitoring. Science 1999;286:531–537. [PubMed: 10521349]

17. Gunel M, Awad IA, Anson J, Lifton RP. Mapping a gene causing cerebral cavernous malformation to 7q11.2-q21. Proc Natl Acad Sci U S A 1995;92:6620–6624. [PubMed: 7604043]

18. Guttmacher AE, Marchuk DA, White RI Jr. Hereditary hemorrhagic telangiectasia. N Engl J Med 1995;333:918–924. [PubMed: 7666879]

19. Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Guttmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA. Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. Nat Genet 1996;13:189–195. [PubMed: 8640225]

20. Jones JM, Bentley E, Meisler MH, Darling SM. Genetic mapping of the voltage-gated shaker potassium channel β subunit Kcnab1 to mouse chromosome 3. Mamm Genome 1998;9:260. [PubMed: 9501317]

21. Kilic T, Pamir MN, Kullu S, Eren F, Ozek MM, Black PMcL. Expression of structural proteins and angiogenic factors in cerebrovascular anomalies. Neurosurgery 2000;46:1179–1192. [PubMed: 10807251]

22. Kim JH. Pathology of epilepsy. Exp Mol Pathol 2001;70:345–367. [PubMed: 11418013]

23. Laberge-le Couteulx S, Jung HH, Labauge P, Houtteville JP, Lescoat C, Cecillon M, Marechal E, Joutel A, Bach JF, Tournier-Lasserve E. Truncating mutations in CCM1, encoding KRIT1, cause hereditary cavernous angiomas. Nat Genet 1999;23:189–193. [PubMed: 10508515]

24. Lee CK, Klopp RG, Weindruch R, Prolla TA. Gene expression profile of aging and its retardation by caloric restriction. Science 1999;285:1390–1393. [PubMed: 10464095]

25. Ling L, Goeddel DV. T6BP, a TRAF6-interacting protein involved in IL-1 signaling. Proc Natl Acad Sci U S A 2000;97:9567–9572. [PubMed: 10920205]

26. Lucas M, Costa AF, Montori M, Solano F, Zayas MD, Izquierdo G. Germline mutations in the *CCM1* gene, encoding Krit1, cause cerebral cavernous malformations. Ann Neurol 2001;49:529–532. [PubMed: 11310633]

27. Matsubara S, Bourdeau A, terBrugge KG, Wallace C, Letarte M. Analysis of endoglin expression in normal brain tissue and in cerebral arteriovenous malformations. Stroke 2000;31:2653–2660. [PubMed: 11062290]

28. McCormick WF. The pathology of vascular ("arteriovenous") malformations. J Neurosurg 1966;24:807–816. [PubMed: 5934138]

29. Mostert V, Selenoprotein P. Properties, functions, and regulation. Arch Biochem Biophys 2000;376:433–438. [PubMed: 10775431]

30. Rigamonti D, Johnson PC, Spetzler RF, Hadley MN, Drayer BP. Cavernous malformations and capillary telangiectasia: A spectrum within a single pathological entity. Neurosurgery 1991;28:60–64. [PubMed: 1994283]

31. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987;238:491–497. [PubMed: 2821619]

32. Sahoo T, Johnson EW, Thomas JW, Kuehl PM, Jones TL, Dokken CG, Touchman JW, Gallione CJ, Lee-Lin SQ, Kosofsky B, Kurth JH, Louis DN, Mettler G, Morrison L, Gil-Nagel A, Rich SS, Zabramski JM, Boguski MS, Green ED, Marchuk DA. Mutations in the gene encoding KRIT, a Krev-1/rap1a binding protein, cause cerebral cavernous malformations (*CCM1*). Hum Mol Genet 1999;8:2325–2333. [PubMed: 10545614]

33. Uranishi R, Awadallah NA, Ogunshola OO, Awad IA. Further study of CD31 protein and messenger ribonucleic acid expression in human cerebral vascular malformations. Neurosurgery 2002;50:110–116. [PubMed: 11844241]

34. Uranishi R, Baev NI, Kim JH, Awad IA. Vascular smooth muscle cell differentiation in human cerebral vascular malformations. Neurosurgery 2001;49:671–680. [PubMed: 11523679]

35. Uranishi R, Baev NI, Ng PY, Kim JH, Awad IA. Expression of endothelial cell angiogenesis receptors in human cerebrovascular malformations. Neurosurgery 2001;48:359–368. [PubMed: 11220380]

36. Van Laer L, Huizing EH, Verstreken M, van Zuijlen D, Wauters JG, Bossuyt PJ, Van de Heyning P, McGuirt WT, Smith RJ, Willems PJ, Legan PK, Richardson GP, Van Camp G. Nonsyndromic

hearing impairment is associated with a mutation in DFNA5. Nat Genet 1998;20:194–197. [PubMed: 9771715]

37. Wong JH, Awad IA, Kim JH. Ultrastructural pathological features of cere-brovascular malformations: A preliminary report. Neurosurgery 2000;46:1454–1459. [PubMed: 10834648]

38. Zhang J, Clatterbuck RE, Rigamonti D, Chang DD, Dietz HC. Interaction between krit1 and ICAP1α infers perturbation of integrin β1-mediated angiogenesis in the pathogenesis of cerebral cavernous malformation. Hum Mol Genet 2001;10:2953–2960. [PubMed: 11741838]

39. Zong Q, Schummer M, Hood L, Morris DR. Messenger RNA translation state: The second dimension of high-throughput expression screening. Proc Natl Acad Sci U S A 1999;96:10632–10636. [PubMed: 10485877]
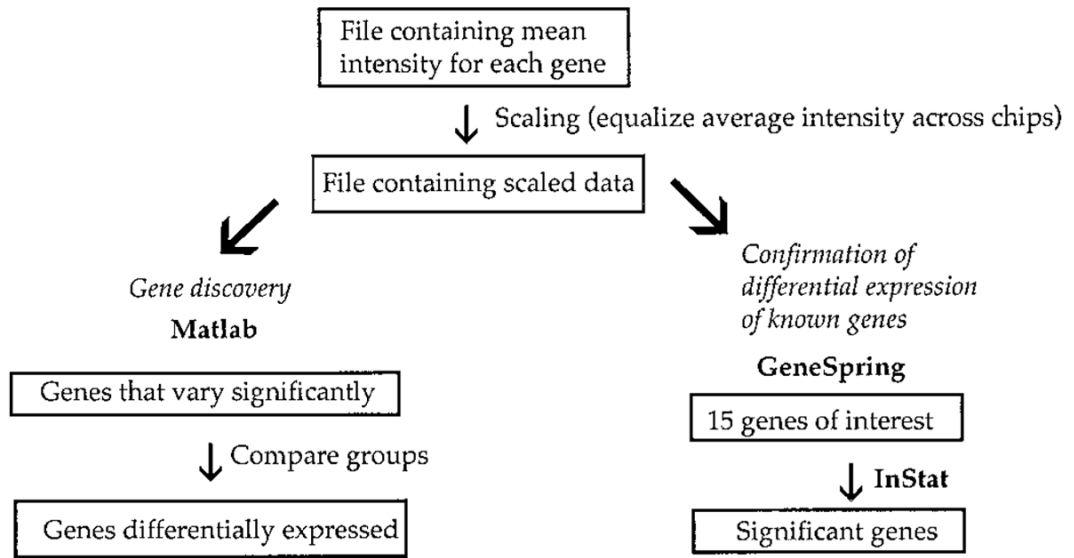
FIGURE 1.
Flow chart illustrating the data analysis of differential gene expression on Affymetrix gene chips. The data were averaged to obtain a mean intensity value of expression for each gene. This mean value was multiplied by a scaling factor to equalize the average intensity across all chips. Files containing scaled data were entered into Matlab version 6.00 release 12 software for gene discovery to identify differentially expressed genes. Files containing scaled data were also entered into GeneSpring software for confirmation analysis. Select genes were chosen and entered into GraphPad InStat version 2.04a program for statistical analysis.
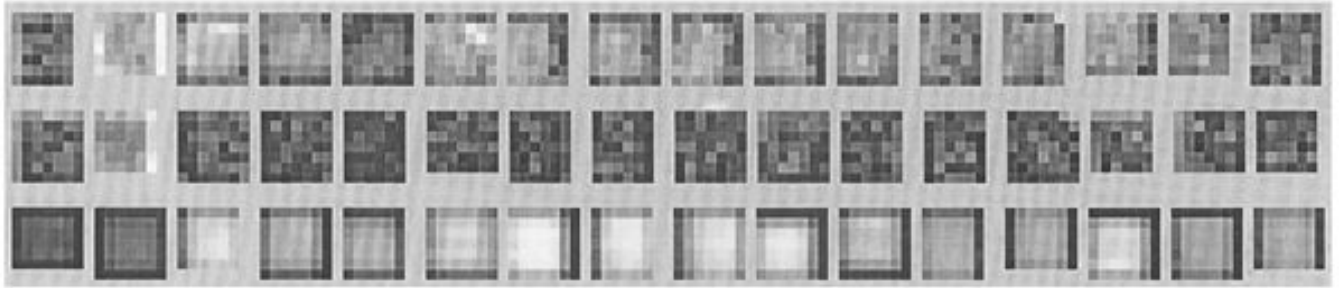
FIGURE 2.
Original scans of the microarray cells containing the 16 perfectly matched oligonucleotide probes for the smooth muscle myosin heavy chain (D10667) hybridized to biotin-labeled cRNA from a representative AVM (*top*), a representative CCM (*center*), and a representative STA (*bottom*). There was a median 87.0-fold decrease when the intensity for all CCMs was compared with the intensities for all AVMs and STAs.
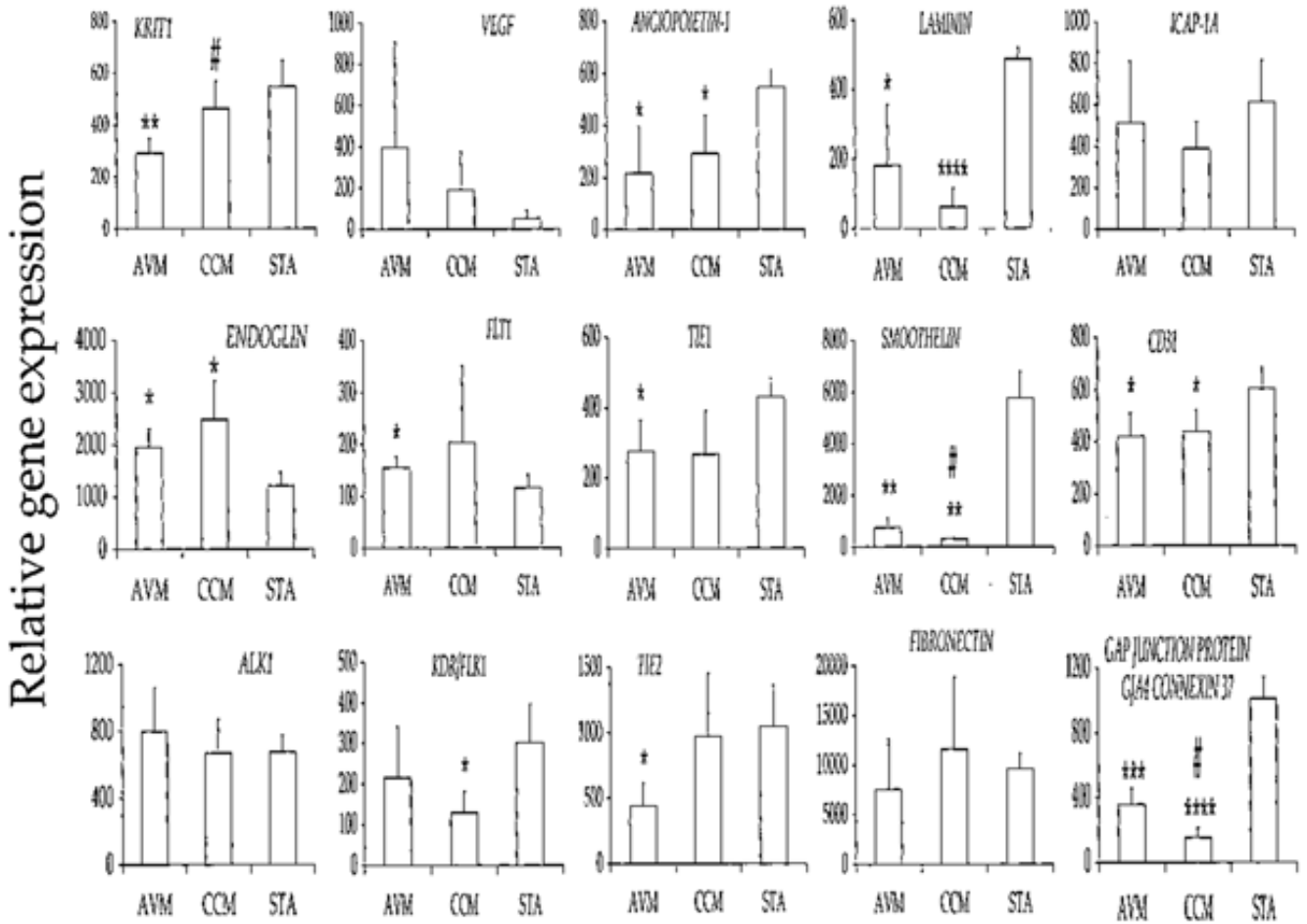
FIGURE 3.
Bar graphs illustrating the differential mRNA expression of 15 relevant proteins identified by immunohistochemistry in AVMs, CCMs, and STAs. Expression is derived by averaging the fluorescence intensity for each gene after scaling chips to an average intensity of 500. *, $P < 0.05$ versus STA; **, $P < 0.01$ versus STA; ***, $P < 0.001$ versus STA; ****, $P < 0.0001$ versus STA; #, $P < 0.05$ versus AVM.

TABLE 1

Summary of patients and lesions[a]

Patient no.	Age (yr)/sex	Diagnosis	Indications for excision	Lesion size (cm)	Lesion location	Fragment weight (g)	Isolated RNA (µg)
1	41/M	AVM	Headache	2.5	L temporal	0.2	5
2	18/F	AVM	Hemorrhage	0.6	L frontal	0.04	1.4
3	28/M	AVM	Hemorrhage	4	L corpus callosum	1	28
4	50/F	AVM	Neurological deficit	4	R temporal	5	246
5	26/F	CCM	Seizure disorder	2.5	L frontal	0.8	30
6	33/F	CCM	Hemorrhage	2.5	L temporal	0.2	50
7	26/M	CCM	Seizure disorder	1.5	R temporal	0.3	29
			Control	1	STA	0.3	5
8	9/F	CCM	Seizure disorder	4	R temporal	2	610
9	68/F	Aneurysm	Control	1	STA	0.3	8.3
10	56/F	Meningioma	Control	1	STA	0.3	4.3

[a]RNA, ribonucleic acid; AVM, arteriovenous malformation; L, left; R, right; CCM, cerebral cavernous malformation; STA, superficial temporal artery.

TABLE 2

Up-regulated genes in cerebral cavernous malformations compared with arteriovenous malformations and healthy superficial temporal arteries[a]

Classification	Accession no.	Fold change	Description
Binding protein	L13210	2.7	Mac-2 binding protein
	AA158243	1.6	FK506-binding protein 2 cis-transpropyl isomerase
Enzyme	U43944	3.2	Breast cancer cytosolic NADP$^+$-dependent malic enzyme
	D17793	2.6	Aldo-keto reductase family 1 member C3
	D86181	2.2	Galactocerebrosidase (Krabbe disease)
	D63998	2.1	Golgi α-mannosidase II
	U09759	2.0	Protein kinase (*MAPK9*)
	L13977	1.8	Prolylcarboxypeptidase
	Y10387	1.6	PAPS synthetase
Immunoglobulin	AF067420	25.8	SNC73 protein
	AI660656	23.3	Like immunoglobulin J chain
	AI932613	21.8	Immunoglobulin-related 14.1 protein precursor
	Y14737	20.4	Immunoglobulin λ heavy chain
	S71043	17.0	Immunoglobulin α heavy chain allotype 2
	M18645	14.8	Immunoglobulin rearranged λ-chain VJC region subgroup λ-IV
	X57809	14.7	Rearranged immunoglobulin λ light chain
	M63438	13.9	Immunoglobulin rearranged λ chain mRNA, VJC region
	X72475	11.9	Rearranged immunoglobulin κ light chain variable region
	AI147237	8.8	Immunoglobulin heavy constant γ 3
	U80114	6.1	Immunoglobulin heavy chain variable region (V4-31)
	AF058075	3.3	Immunoglobulin λ light chain VJ region
	X05323	1.6	MRC OX-2 membrane glycoprotein
Membrane protein	M16276	42.7	DQw1-βMHC Class II HLA-DR2-Dw12
	AB023204	3.3	Erythrocyte membrane protein band 4.1-like 3
	J04182	1.4	Lysosomal membrane glycoprotein-1 (*LAMP1*)
Miscellaneous	AF073308	3.7	Nonsyndromic hearing impairment protein 5 (*DFNA5*)
	Z11793	3.7	Selenoprotein P
	U03877	3.0	Extracellular matrix S1-5 epidermal growth factor-containing fibulin
	AF055376	2.8	V-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
	M63835	2.4	Immunoglobulin G Fc receptor I gene
	U68494	2.2	Hbc647 from pancreatic islet
	D87075	2.0	Solute carrier family 23 (nucleobase transporters), member 1
	AB023176	1.9	Ral GTP dissociation stimulator-like protein (signaling)
	L13939	1.8	β adaptin (transport) (*AP1B1*)
	X99584	1.6	SMT3A similar to suppressor of MIF 2
	D26599	1.5	Proteasome subunit (*PSMB2*)
Unknown	AB018259	3.0	KIAA0716 large protein from brain
	H24861	2.1	3NbHBst gene from Soares breast

Classification	Accession no.	Fold change	Description
	AL050084	1.8	Hypothetical protein DKFZp566O1646
	AF035313	1.6	Hypothetical protein 23851
	AF055029	1.6	Hypothetical gene 24711
	AL050197	1.5	Hypothetical protein DKFZp586D0623

[a]NADP$^+$, oxidized nicotinamide adenine dinucleotide phosphate; PAPS, adenosine 3′-phosphate 5′-phosphosulfate; VJC, variable joining constant; VJ, variable joining; mRNA, messenger ribonucleic acid; MHC, major histocompatibility complex; MIF, mitotic fidelity of chromosome transmission protein.

TABLE 3

Down-regulated genes in cerebral cavernous malformations compared with arteriovenous malformations and healthy superficial temporal arteries

Classification	Accession no.	Fold change	Description
Apoptosis	U83981	2.5	Protein phosphatase 1, regulatory (inhibitor) subunit 15A
	Z35491	1.4	BCL2 associated athanogene-1 glucocorticoid receptor-associated protein
Cellular components	D17408	22.8	Calponin 1, basic smooth muscle
	M63391	15.2	Desmin
	J00068	8.7	Adult skeletal muscle α-actin
	L38486	4.6	Microfibril-associated glycoprotein 4
	M77016	4.6	Tropomodulin tropomyosin binding protein
	AI888563	3.6	Smoothelin
	M95787	2.9	Transgelin 22-kDa smooth muscle protein
	AL050396	2.3	Filamin A endothelial actin binding protein
	X13839	2.2	Vascular smooth muscle α-actin
Enzyme	J03779	1.9	Membrane metalloendopeptidase (*MME*)
	U43195	1.8	ρ-associated, coiled-coil containing protein kinase (*ROCK1*)
	AL049415	1.5	Disintegrin and metalloproteinase 19 (meltrin β)
	AF038203	1.4	LIS1-interacting protein NUDEL, endooligopeptidase A
	AF045458	1.4	Serine/threonine kinase (*ULK1*)
Heat shock protein	Z23090	2.0	Heat shock 27-kDa protein 1
	M16660	1.3	Heat shock 90-kDa protein 1, β
Myosin	D10667	87.0	Smooth muscle myosin heavy chain
	AF013570	20.4	Smooth muscle myosin heavy chain SM2
	AF001548	17.8	Myosin heavy chain (*MHY11*)
	M12125	6.7	Fibroblast muscle-type tropomyosin
	J02854	5.6	Myosin light chain (*MYL9*)
	M22919	2.2	Nonmuscle/smooth muscle alkali myosin light chain
Miscellaneous	U49957	3.3	LIM protein (*LPP*)
	M96789	3.1	Connexin 37 gap junction protein (*GJA4*)
	U66579	2.7	G protein-coupled receptor-20
	M35999	2.5	Integrin β chain platelet glycoprotein IIIa
	X67055	2.5	Inter-α-trypsin inhibitor heavy chain H3
	AF035119	1.8	Deleted in liver cancer-1 (*DLC1*)
	AF032886	1.8	Forkhead protein (*FOXO3A*)
	M88279	1.3	Immunophilin (*FKBP4*) binding protein
	U25789	1.3	Ribosomal protein L21
Unknown	AB011099	9.5	KIAA0527 large protein from brain
	AB023139	4.4	KIAA0922 large protein from brain
	AB028999	1.5	KIAA1076 large protein from brain

TABLE 4

Up-regulated genes in arteriovenous malformations compared with cerebral cavernous malformations and healthy superficial temporal arteries[a]

Classification	Accession no.	Fold change	Description
Enzyme	AF016833	8.0	Maltase-glucoamylase (α glucosidase)
	D10495	2.8	Protein kinase C δ-type
	N36295	2.0	Dolichyl-phosphate mannosyltransferase
	U50648	1.9	Double-stranded RNA-dependent protein kinase
	U89896	1.7	Casein kinase I γ 2
	HG1614-HT1614	1.5	Protein phosphatase 1, α catalytic subunit
	M91029	1.5	AMP deaminase
	AJ012590	1.4	Glucose 1-dehydrogenase
Nuclear	S77763	6.3	Nuclear factor erythroid-derived 2
	L17131	2.0	High-mobility group protein (*HMGIY*)
	Z80780	2.0	Histone gene (*H2BFH*)
	AI688098	1.8	H2B histone family, member 2
	M58603	1.7	Nuclear factor κB DNA binding subunit
Receptor	U11872	17.0	Interleukin-8 receptor type B (*IL8RB*)
	D12763	10.4	ST2 interleukin 1 receptor-like 1 protein
	L19593	7.5	Interleukin 8 receptor β
	U43672	6.1	Interleukin 18 receptor 1
	AC004262	4.8	EMR2 epidermal growth factor-like
	M59818	4.5	Granulocyte colony-stimulating factor receptor
	U11870	4.1	Interleukin-8 receptor type A
	AF014794	3.3	TNF-related receptor (*TNFRSF10C*)
	AJ000479	2.8	Endothelial G-protein coupled receptor 6
	M59820	2.2	Granulocyte colony-stimulating factor receptor
	M84562	2.2	Formyl peptide receptor-like receptor (*FPRL1*)
Ubiquitin	U49869	1.5	Ubiquitin B
	X04803	1.5	Ubiquitin B
	AB009010	1.4	Ubiquitin C
	AL050254	1.3	F-box only protein 7 of SCF-ubiquitin-ligase
Miscellaneous	D85429	4.6	Heat shock protein 40 (*DNAAJB1*)
	U49187	4.5	PL48 cytotrophoblast differentiation
	V00505	2.7	δ-globin
	X03656	2.3	Granulocyte colony-stimulating factor
	HG2259-HT2348	2.0	Tubulin, α 1, isoform 44
	AI985964	1.8	Intestinal trefoil factor-like
	L40393	1.8	S171 NUMB homolog, Alzheimer's disease locus
	U12707	1.8	Wiskott-Aldrich syndrome protein
	L22005	1.7	Cell division cycle 34
	M92287	1.6	Cyclin D3
	S62138	1.6	DNA-damage-inducible transcript 3 (*DDIT3*)
	M33509	1.5	HLA-B-associated transcript 2

Classification	Accession no.	Fold change	Description
	AF053356	1.4	Insulin receptor substrate 3-like protein
	S78771	1.4	Bromodomain-containing protein 2
	Z19574	1.4	Cytokeratin 17 intermediate filament protein
	AF089750	1.2	Fotillin-1 caveolae-associated membrane protein
Unknown	AB002384	5.4	KIAA0386 large protein from brain
	X90579	3.0	DNA for cyp-related pseudogene
	AB002344	2.4	KIAA0346 large protein from brain
	R48209	1.9	Protein KIAA1539 from Soares breast

[a]RNA, ribonucleic acid; AMP, adenosine monophosphate; DNA, deoxyribonucleic acid; TNF, tumor necrosis factor.

TABLE 5

Down-regulated genes in arteriovenous malformations compared with cerebral cavernous malformations and healthy superficial temporal arteries[a]

Classification	Accession no.	Fold change	Description
Apoptosis	AF035606	1.7	Calcium-binding programmed cell death protein 6
	AB020680	1.6	BCL2-associated athanogen 5 protein
Binding protein	AL080119	1.7	Plasminogen activator inhibitor-1 mRNA binding protein (*PAI-RBP1*)
	U41654	1.7	Ras-related GTP-binding protein (*RAGA*)
	X81789	1.7	RNA-binding splicing factor (*SF3A60*)
	X63753	1.6	SON DNA binding protein
	AJ132258	1.4	Staufen double-stranded RNA binding protein
Cell adhesion	X53586	1.9	Integrin α 6
	L23805	1.8	α 1(E)-catenin
	D14705	1.5	α-catenin cahedrin-associated tight cell-cell adhesion protein
	M35011	1.5	Integrin β-5 subunit
Enzyme	X96752	3.0	L-3-hydroxyacyl-CoA dehydrogenase
	M37721	2.6	Peptidylglycine α-amidating monooxygenase
	X58288	2.4	Protein tyrosine phosphatase
	M12267	2.1	Ornithine aminotransferase
	AB018262	1.8	Translocase of outer mitochondrial membrane 70
	M31899	1.8	DNA repair helicase (*ERCC3*)
	D37931	1.5	RNase 4
	AA203303	1.3	NADH dehydrogenase (ubiquinone) Fe-S protein 4
Oncology related	AB002450	2.3	CGI-109 protein, deleted in advanced lung cancer
	L13689	2.1	Proto-oncogene (*BMI1*)
	Z50022	1.5	Pituitary tumor-transforming 1 interacting surface glycoprotein
	U41635	1.2	Amplified in sarcomas (*OS-9*)
Receptor ligand	X57025	7.2	Insulin-like growth factor I
	L36033	4.2	Pre-B cell stimulating factor homolog (SDF1b) chemokine
Transcriptional regulation	U14193	2.0	Transcriptional factor TFIIA γ subunit
	M81601	1.7	Transcription elongation factor (SII)
	U71267	1.7	Potential transcriptional repressor (*CNOT4*)
Translation initiation factor	D30655	1.4	Eukaryotic initiation factor 4AII
	AF035280	1.3	Translation initiation factor 2B
Transporter	U81375	2.1	Equilibrative nucleoside transporter 1
	U77942	1.6	Syntaxin 7 vesicle trafficking to lysosomes
Miscellaneous	AB007865	2.8	Fibronectin leucine-rich transmembrane protein 2
	AI087268	2.4	Small nuclear ribonucleoprotein polypeptide C
	L08044	2.4	Intestinal trefoil secretory protein
	U17077	2.3	BENE proteolipid protein
	U41816	2.1	C-1 prefolen 4 chaperone
	D16217	2.0	Calpastatin

Classification	Accession no.	Fold change	Description
	AL031177	1.9	26S proteasome subunit p28 and collagen α 6
	D11428	1.8	Peripheral myelin glycoprotein (*PMP22*)
	J04543	1.8	Synexin voltage-dependent calcium channel
	U18291	1.8	Cell division cycle 16 (*CDC16*)
	X68194	1.8	Synaptophysin-like protein (*SYPL*)
	AB028964	1.6	Putative forkhead domain
	AB002332	1.6	Clock homolog circadian rhythm gene
	U90913	1.6	Tax interaction protein-1
Unknown	AL080234	3.0	FBD3 cri-du-chat critical region
	AI760932	2.5	CGI-60 protein
	AA115140	2.0	KIAA1354 large protein from brain
	AL080063	1.9	DKFZp564I052 protein
	N53547	1.9	Hypothetical protein MGC5508
	AF035313	1.7	Hypothetical protein 23851
	D63477	1.7	KIAA0143 gene
	AL049944	1.6	DKFZp564G2022 protein
	AL049957	1.6	DKFZp564J0323 protein
	AB011164	1.5	KIAA0592 large protein from brain
	AL035304	1.5	DKFZP564B167 gene
	AB028972	1.4	KIAA1049 large protein from brain
	AB014597	1.3	KIAA0697 large protein from brain

[a] mRNA, messenger ribonucleic acid; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; CoA, coenzyme A; NADH, nicotinamide adenine dinucleotide.