**EPHB4** Gene Polymorphisms and Risk of Intracranial Hemorrhage in Patients With Brain Arteriovenous Malformations

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**Background**—Brain arteriovenous malformations (BAVMs) are a tangle of abnormal vessels directly shunting blood from the arterial to venous circulation and an important cause of intracranial hemorrhage (ICH). EphB4 is involved in arterial-venous determination during embryogenesis; altered signaling could lead to vascular instability resulting in ICH. We investigated the association of single-nucleotide polymorphisms (SNPs) and haplotypes in **EPHB4** with risk of ICH at clinical presentation in patients with BAVM.

**Methods and Results**—Eight haplotype-tagging SNPs spanning ∼29 kb were tested for association with ICH presentation in 146 white patients with BAVM (phase I: 56 ICH, 90 non-ICH) using allelic, haplotypic, and principal components analysis. Associated SNPs were then genotyped in 102 additional cases (phase II: 37 ICH, 65 non-ICH), and data were combined for multivariable logistic regression. Minor alleles of 2 SNPs were associated with reduced risk of ICH presentation (rs314313_C, \( P = 0.005 \); rs314308_T, \( P = 0.0004 \)). Overall, haplotypes were also significantly associated with ICH presentation (χ² = 17.24, 6 df; \( P = 0.008 \)); 2 haplotypes containing the rs314308 T allele (GCCTGGGT, \( P = 0.003 \); GTCTGGGC, \( P = 0.036 \)) were associated with reduced risk. In principal components analysis, 2 components explained 91% of the variance and complemented haplotype results by implicating 4 SNPs at the 5’ end, including rs314308 and rs314313. These 2 SNPs were replicated in the phase II cohort, and combined data resulted in greater significance (rs314313, \( P = 0.0007 \); rs314308, \( P = 0.00008 \)). SNP association with ICH presentation persisted after adjusting for age, sex, BAVM size, and deep venous drainage.

**Conclusions**—**EPHB4** polymorphisms are associated with risk of ICH presentation in patients with BAVM, warranting further study. ([Circ Cardiovasc Genet. 2009;2:476-482.](http://circgenetics.ahajournals.org/ci125257.html))

**Key Words:** cerebrovascular disorders ■ genetics ■ hemmorhage ■ receptors ■ risk factors

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**Clinical Perspective on p 482**

**EPHB4** encodes the EPH (erythropoietin-producing hepatocellular) receptor B4 (protein abbreviation, EphB4), a tyrosine kinase receptor expressed in venous endothelial cells. As the cognate receptor for the arterial endothelial cell ligand ephrinB2 (encoded by **EFNB2**), EphB4 plays an important role in embryonic vascular development, especially in arterial-venous determination. Mutant mice that lack Ephb4 or Efnb2 die at embryonic day 9.5 as a result of defective angiogenic remodeling and vasculogenesis. The primitive blood vessels form; however, the primary vascular plexus fails to develop into a hierarchical system of arterial and venous networks. Further study of this intriguing pathway may provide novel insights into the pathogenesis and treatment of BAVM hemorrhage.

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large vessels and capillaries, resulting in a phenotype resembling BAVMs. Recently, a mouse model of perinatal brain arteriovenous fistula formation suggested that Notch and ephrinB2/EphB4 signaling pathways are essential for balanced arteriovenous development during blood vessel formation.\textsuperscript{13} EphrinB2/EphB4 signaling also regulates blood vessel morphogenesis and patterning of the postnatal vascular system, and functions in blood vessel permeability, inflammation, wound healing and pathological (tumor) angiogenesis.\textsuperscript{14–18} Vascular endothelial growth factor and Notch pathways influence venous \textit{EPHB4} gene expression\textsuperscript{19–20}; thus, altered EphB4 signaling could affect the integrity of the vascular wall eventually leading to rupture. The purpose of this study was to investigate whether polymorphisms in the \textit{EPHB4} gene are associated with ICH risk at initial presentation in patients with BAVM.

**Methods**

**Patient Population**

This was a cross-sectional study of white adult patients with BAVM. The main group factor was whether or not the patients presented initially with ICH. ICH presentation was defined as new intracranial blood on computed tomography or MRI. All other presentations without evidence of new bleeding, including seizure, focal ischemic deficit, and headache, apparently unrelated symptoms, or asymptomatic, incidental discovery were coded as unruptured. BAVM cases were recruited at the University of California, San Francisco (UCSF) or at Kaiser Permanente Medical Care Program of Northern California (KPMCP),\textsuperscript{21} and classified using standardized guidelines.\textsuperscript{22} The study was approved by the Institutional Review Boards of UCSF and KPMCP, and all subjects provided written, informed consent, and blood or saliva specimens for genetic studies.

The study was conducted in 2 phases. In phase I, unrelated white BAVM cases (\(n=236\); 90 ICH, 146 non-ICH) from our larger prospective BAVM registry were genotyped for 8 haplotype-tagging single-nucleotide polymorphisms (SNPs) in \textit{EPHB4}. One hundred and forty-six patients with BAVM (56 ICH, 90 non-ICH) were successfully genotyped for all 8 SNPs and were included in the phase I cohort (haplotypic and principal components [PCs] analyses described below required that all patients have genotypes for all 8 SNPs). Of the 90 patients excluded from phase I due to missing genotype data for one or more of the 8 SNPs, 63% of these (\(n=57\)) were subsequently included in the phase II cohort with complete data for the 2 significantly associated SNPs (Bonferroni corrected \(P<0.0063\)) along with 45 newly recruited patients (\(n=102\) total patients, 37 ICH, 65 non-ICH). Subsequently, a joint analysis of 248 subjects was performed. To minimize the possibility of population stratification confounding our results, we included only white subjects in all analyses.

**Polymorphism Selection and Genotyping**

We selected 8 haplotype-tagging SNPs (2 exonic, 4 intronic, and 2 intergenic) for a \(\sim 29\) kb region encompassing the \textit{EPHB4} gene. Using data from the HapMap project (http://hapmap.org), SNPs with a minor allele frequency \(>2\%\) in the white CEU or Han Chinese in Beijing (CHB) samples were selected using the Tagger algorithm\textsuperscript{23} implemented in Haploview (dbSNP build 125 on NCBI human genome build 35),\textsuperscript{24} with pairwise selection and \(r^2>0.8\). Genomic DNA was extracted from peripheral blood lymphocytes using a salt modification method (Gentra Systems, Minneapolis, Minn). Polymorphism-spanning fragments were amplified by polymerase chain reaction and genotyped by Beckman-Coulter SNPstream 48plex technology or by template-directed primer extension with fluorescence polarization detection.\textsuperscript{25} Genotyping was performed by investigators blinded to clinical status.

**Statistical Analysis**

**Single Marker Association**

SNP and haplotype association analyses were carried out using the software package \textit{plink} version 1.01 (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml).\textsuperscript{26} Individual SNPs were screened for association with ICH using the 1-deg allic \(\chi^2\) test. Odds ratios (ORs) and 95% CIs were calculated for each SNP. Correction for multiple testing was applied using the Bonferroni method. Nominal probability values are reported with a corrected threshold for significance of \(P=0.0063\) (\(P=0.05/8\)).

When the true genetic model is not known, the codominant or additive genetic model (with fewer df) has been recommended.\textsuperscript{27} Therefore, to limit the number of tests and reduce the df for that test, all statistical analyses were performed assuming an additive genetic model, unless otherwise stated. Genetic variants significantly associated with ICH were further evaluated in a joint analysis of 248 patients using Intercooled Stata 9 statistical software (Stata Corp, College Station, TX). Logistic regression analysis was performed for different genetic models (codominant, dominant, and additive), with further adjustments for age, sex (female versus male), and recruitment site (UCSF versus KPMCP). Additional adjustments for BAVM size and deep venous drainage were performed for the subset of 180 patients with complete morphological data.

**Haplotype Association**

Eight-SNP fixed and 3-SNP sliding window haplotype frequencies were inferred using the expectation-maximization algorithm. Both a global likelihood ratio test of association comparing the overall haplotype distribution between ICH and non-ICH BAVM cases, and haplotype-specific tests of association comparing each haplotype versus all other haplotypes were performed. Degrees of freedom are equal to number of haplotypes tested minus 1 and significance was set at \(P<0.05\). Only common haplotypes with a minor allele frequency greater than 1% were considered for analysis.

**Principal Components Analysis**

Principal Components analysis has been proposed as an alternative method for testing disease and SNP associations. This approach captures the linkage disequilibrium (LD) information within a candidate region without the need to predict haplotypes or to determine haplotype blocks.\textsuperscript{28} Briefly, this method reduces the number of correlated SNPs into uncorrelated linear composite variables (principal components) that explain most of the variance.

Using phase I data, we performed principal components analysis of the covariance matrix of 8 SNPs, with each SNP coded as having 0, 1, or 2 copies of the minor allele (additive genetic model) (Stata 9). Principal components meeting a threshold of \(>0.80\) variance explained were retained, as the small amount of variance explained by additional principal components does not provide enough information to justify the additional df required to include them.\textsuperscript{29} Principal components were included as predictor variables in a test of association with ICH presentation, using logistic regression analysis. A significant association of principal components implies that the combination of SNPs in the principal component is associated with ICH. SNPs with larger factor loadings in the principal components are interpreted as explaining the majority of the variation among the linear combination of SNPs.

**Linkage Disequilibrium**

The strength of LD between SNPs at the \textit{EPHB4} locus was estimated by computing the correlation coefficient \(r^2\) in Haploview using all genotype data from the 248 patients with BAVM.\textsuperscript{20} An \(r^2\) value of 1 indicates perfect LD (complete correlation), whereas an \(r^2\) value of 0 indicates no LD.

**Case-Control Analysis**

We also conducted a secondary case-control analysis of all 248 patients with BAVM (93 ICH and 155 non-ICH) and 225 healthy controls of self-reported white ancestry. Controls were healthy volunteers from the same clinical catchment area without significant medical history recruited for a pharmacogenetics study conducted at...
Table 1. Clinical Characteristics of BAVM Patients With and Without ICH at Presentation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ICH (n=93)</th>
<th>Non-ICH (n=155)</th>
<th>Total (n=248)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, y</td>
<td>34.4±17.6</td>
<td>40.8±16.6</td>
<td>38.4±17.2</td>
<td>0.005*</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47 (50.5)</td>
<td>69 (44.5)</td>
<td>116 (46.8)</td>
<td>0.358</td>
</tr>
<tr>
<td>Female</td>
<td>46 (49.5)</td>
<td>86 (55.5)</td>
<td>132 (53.2)</td>
<td></td>
</tr>
<tr>
<td>BAVM size, cm†</td>
<td>2.6±1.5</td>
<td>3.1±1.5</td>
<td>2.9±1.5</td>
<td>0.031*</td>
</tr>
<tr>
<td>Deep venous drainage†</td>
<td>Yes</td>
<td>18 (23.1)</td>
<td>19 (15.3)</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>60 (76.9)</td>
<td>105 (84.7)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%). *P value calculated by t test; all others were calculated by χ² test. †Counts do not add up to total because of missing data. A total of 180 patients had complete phenotypic data.

UCSF.30 Assuming an additive genetic model, logistic regression analysis was performed to obtain ORs and 95% CIs for SNPs rs314313 and rs314308, adjusting for age and sex.

Phase I Analyses

We genotyped 8 haplotype-tagging SNPs located in the EPHB4 gene in 56 ICH and 90 non-ICH BAVM cases. All SNPs were polymorphic (minor allele frequency >1%), and genotype frequencies (Table 2) differed significantly between ICH and non-ICH cases for rs314353 (P=0.010), rs314308 (P=0.006), and rs314313 (P=0.012). Allelic association analysis identified 4 markers nominally associated with ICH presentation (P<0.05, Table 3). The minor alleles of 2 SNPs remained significantly associated with reduced risk of ICH presentation after Bonferroni correction: rs314313 (C; OR, 0.45; 95% CI, 0.25 to 0.79) and rs314308 (T; OR, 0.36; 95% CI, 0.20 to 0.65). These two SNPs, located in intron 1 and 3, were in high LD (r²=0.88, Figure) and located in the same LD block.

Next, we performed haplotype analyses in attempt to refine the association signal. Overall, 7 common haplotypes were predicted with frequencies between 2% and 37%. A global test of association comparing the overall haplotype distribution between ICH and non-ICH cases was significant (χ²=17.24, df=6, P=0.008). Two haplotypes containing the minor allele of rs314308 (T) were associated with reduced risk (GCC7GGGT, P=0.003; and GTCTGGGC, P=0.036) of ICH presentation (Table 4). The more common haplotype (GCC7GGGT, frequency of ICH, 0.157; frequency in non-ICH, 0.318) was consistent with the individual SNP analysis, as it contains the minor alleles for the 2 significantly associated SNPs. Sliding windows of 3-SNP haplotypes excluded SNPs rs314346 and rs314353, as the first 2 windows including these SNPs were not associated with ICH status (data not shown).

To complement haplotype analysis, we performed principal components analysis, which identified 8 principal components. The first 2 principal components explained 91% of the total variance in the locus. The third principal component explained 5.4% of the variance, whereas the remaining 5 principal components together explained only 3.7% of the total variance. Therefore, only the first 2 principal components were retained for further analysis.
The first PC explained 56.9% of the variance, and all 8 SNPs had approximately equal factor loadings (Table 5). The second PC explained an additional 34.1% of the variance, with the 4 SNPs at the 5' end of *EPHB4* having higher factor loadings (45%). rs314313 and rs314308 had positive factor loadings whereas rs2247445 and rs2250818 had negative factor loadings, suggesting that the minor alleles present on positively loading SNPs correlated with major alleles on negatively loading SNPs on PC2. Both PC1 (*P*=0.013) and PC2 (*P*=0.021) were independently associated with ICH when used directly as predictor variables for ICH presentation in a logistic regression model.

Joint Analysis

To replicate the findings, SNPs rs314313 and rs314308 were genotyped in a phase II cohort of BAVM cases (37 ICH, 65 non-ICH). Phase I and phase II cases were similar with respect to age, sex, hemorrhagic status at initial presentation, BAVM size, and deep venous drainage (Supplemental Table). Allele and genotype frequencies were similar in both cohorts. Both SNPs were associated with ICH presentation in the phase II cohort (rs314313: OR, 0.53; 95% CI, 0.28 to 1.00; *P*=0.051; rs314308: OR, 0.54; 95% CI, 0.29 to 1.00; *P*=0.050) with risk estimates similar to those observed in the phase I cohort presented in Table 3. We then evaluated both cohorts together as a combined dataset, which included 93 ICH and 155 non-ICH cases. SNPs rs314313 (OR, 0.48; 95% CI, 0.31 to 0.74; *P*=0.0007) and rs314308 (OR, 0.43; 95% CI, 0.28 to 0.66; *P*=0.00008) were significantly associated with ICH. The codominant and dominant models for the 2 associated SNPs are presented in Table 6. Compared with the homozygote major allele group, the OR for the heterozygote and homozygote minor allele groups suggest an additive effect, with risk estimates approximately halving for each copy of the minor allele.

Multivariable logistic regression analysis, assuming an additive genetic model and adjusting for age, sex, and recruitment site in the combined cohort, yielded similar results for rs314313 and rs314308.

Table 3. Phase I Allelic Association of *EPHB4* Polymorphisms With ICH Presentation

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>ICH MAF</th>
<th>Non-ICH MAF</th>
<th>OR</th>
<th>95% CI</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>rs314346_C</td>
<td>0.571</td>
<td>0.444</td>
<td>1.67</td>
<td>1.04 to 2.68</td>
<td>0.0348</td>
</tr>
<tr>
<td>rs314353_A</td>
<td>0.509</td>
<td>0.367</td>
<td>1.79</td>
<td>1.11 to 2.89</td>
<td>0.0167</td>
</tr>
<tr>
<td>rs2230585_A</td>
<td>0.429</td>
<td>0.339</td>
<td>1.46</td>
<td>0.90 to 2.38</td>
<td>0.1234</td>
</tr>
<tr>
<td>rs144173_A</td>
<td>0.464</td>
<td>0.350</td>
<td>1.61</td>
<td>0.99 to 2.61</td>
<td>0.0520</td>
</tr>
<tr>
<td>rs314308_T</td>
<td>0.170</td>
<td>0.361</td>
<td>0.36</td>
<td>0.20 to 0.65</td>
<td>0.0004*</td>
</tr>
<tr>
<td>rs2250818_T</td>
<td>0.313</td>
<td>0.250</td>
<td>1.36</td>
<td>0.81 to 2.30</td>
<td>0.2443</td>
</tr>
<tr>
<td>rs314313_C</td>
<td>0.179</td>
<td>0.328</td>
<td>0.45</td>
<td>0.25 to 0.79</td>
<td>0.0053*</td>
</tr>
<tr>
<td>rs2247445_A</td>
<td>0.313</td>
<td>0.239</td>
<td>1.45</td>
<td>0.86 to 2.45</td>
<td>0.1669</td>
</tr>
</tbody>
</table>

MAF indicates minor allele frequency.

*Significant after Bonferroni correction at *P*<0.0063.

Table 4. Phase I *EPHB4* Fixed Window Haplotype Association With ICH

<table>
<thead>
<tr>
<th>Haplotype (5'→3')</th>
<th>Frequency Estimates</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICH</td>
<td>Non-ICH</td>
</tr>
<tr>
<td>Global <em>P</em> value</td>
<td></td>
<td>0.008</td>
</tr>
</tbody>
</table>

Specific haplotypes

- GCTGG6GT: 0.157, 0.318, 0.003
- GCTGG6GC: 0.000, 0.040, 0.036
- ATTCGGAC: 0.046, 0.011, 0.065
- GTCCAAAC: 0.417, 0.330, 0.138
- GTCCGGGT: 0.278, 0.233, 0.397
- GTCCGGGC: 0.065, 0.045, 0.479
- GTCCAGAC: 0.037, 0.023, 0.479

*Haplotype specific *P* value is a comparison of each individual haplotype with all other haplotypes.
(OR, 0.51; 95% CI, 0.33 to 0.79; \( P = 0.002 \)) and for rs314308 (OR, 0.49; 95% CI, 0.32 to 0.74; \( P = 0.001 \)). Further adjustments for BAVM size and deep venous drainage in the subset of 180 patients with complete morphological data also did not change results (rs314313: OR, 0.53; 95% CI, 0.32 to 0.88; \( P = 0.014 \); rs314308: OR, 0.48; 95% CI, 0.29 to 0.79; \( P = 0.004 \)).

Case-Control Analyses

To determine whether SNPs rs314313 and rs314308 were also associated with BAVM disease susceptibility, we performed a case-control analysis. Genotype frequencies for white controls (rs314313: TT, 41.3%; CT, 48.5%; CC, 10.2%; rs314308: CC, 41.3%; CT, 43.1%; TT, 15.6%) were similar to BAVM cases reported in Table 2, and both SNPs were in HWE in controls. However, controls were younger than cases (30.5 \( \pm \) 5.7 years versus 38.4 \( \pm \) 17.2 years, \( P < 0.001 \)), with a similar sex distribution (42.7% versus 46.8% male, \( P = 0.370 \)).

When all 248 BAVM cases (ICH and non-ICH combined) were compared with controls in an additive genetic model adjusting for age and sex, SNPs rs314308 (OR, 0.76; 95% CI, 0.58 to 0.99; \( P = 0.046 \)) and rs314313 (OR, 0.76; 95% CI, 0.57 to 1.02; \( P = 0.066 \)) were marginally associated with reduced risk of BAVM. However, the overall reduced risk seemed to be driven by the difference between the 93 ruptured BAVM cases and controls (rs314313: OR, 0.50; 95% CI, 0.33 to 0.77; \( P = 0.002 \); rs314308: OR, 0.48; 95% CI, 0.32 to 0.72; \( P < 0.001 \)). SNPs were not associated with unruptured BAVM risk when 155 non-ICH cases were compared with controls (rs314313: OR, 0.94; 95% CI, 0.67 to 1.32; \( P = 0.713 \); rs314308: OR, 0.95; 95% CI, 0.70 to 1.30; \( P = 0.746 \)). These findings suggest the SNP association results are specific to ICH presentation and not BAVM status.

Discussion

We provide the first report of an association between polymorphic variants in the *EPHB4* gene with risk of ICH at presentation in patients harboring BAVM. Using 3 different statistical approaches, we identified 2 SNPs (rs314313 and rs314308) located proximal to the 5’ end of the *EPHB4* gene that contribute to a 50% to 60% reduction in risk of ICH in patients with BAVM who carry the minor alleles. Furthermore, case-control analyses support the findings that these 2 SNPs influence hemorrhagic risk, but not BAVM risk.

The 2 associated *EPHB4* SNPs are located in intronic regions not well conserved with no known function. Hence, they are likely not causal alleles, but surrogate markers in LD with functional polymorphisms located elsewhere in the *EPHB4* gene or closely neighboring gene. Interactions between *EPHB4* and genes that map to the same chromosomal region (\( \pm 10 \) Mb) have not been reported. However, there are examples in the literature of noncoding sequences functioning as gene regulatory elements. The primary transcript of the *EPHB4* gene is alternatively spliced. However, the 2 intronic polymorphisms found to be associated with reduced risk of ICH presentation in patients with BAVM are not located near a splice site; thus are not likely to influence splicing efficiency. The SNPs are located adjacent to exons that encode the extracellular ligand binding domain. One explanation for our findings is that these SNPs may be in disequilibrium with other *EPHB4* SNPs located in exons that may be protective of ICH by affecting *EPHB4* gene or encoded protein expression, or influencing receptor-ligand binding. Changes in ephrinB2/EphB4 interaction may have an effect on vessel wall stability and response to shear stress that could make vessels prone to hemorrhage.

The role of Eph receptors and their ligands in vascular function became apparent when genetic loss-of-function experiments revealed ephrinB2 and its receptors (EphB2, EphB3, and EphB4) control arteriovenous assembly and differentiation during development; both *ephb4* \( ^{−/−} \) and *epfb2* \( ^{−/−} \) embryos suffer from severe vascular phenotypes including fatal abnormalities of capillary formation. Eph receptor-ligand signaling is not limited to vascular development and has been implicated in adult vascular biology, including in tumor angiogenesis and progression, and more recently in monocyte adhesion and transmigration through the vascular endothelium. Additional studies have implicated ephrin/Eph receptor interactions in inflammation.
Inflammation contributes to the pathogenesis of several vascular malformations including cerebral cavernous malformations, intracranial aneurysms, and abdominal aortic aneurysms. Recent studies have demonstrated the presence of inflammatory cells (neutrophils and macrophages) in BAVM tissue, suggesting a role for inflammation in BAVM disease progression and rupture. Additionally, we have previously reported an association of the IL6-174 GG genotype with BAVM hemorrhagic presentation, and IL-1β promoter polymorphisms associated with increased risk of subsequent ICH and BAVM susceptibility, further implicating inflammatory processes in BAVM rupture.

Although ephrins and Eph receptors are fundamentally involved in embryonic vascular development, it is now known that they are abundantly expressed in both endothelial and epithelial cells in adult mammals, and studies suggest that Eph receptors may play a role in inflammation by regulating the permeability of endothelial and epithelial barriers. Rat models have shown that during later stages of inflammation there is a decrease in the expression of several Eph receptors, including EphB4, on leukocytes and endothelial cells, promoting adhesion of leukocytes to endothelial cells. These reports suggest that EphB4 could play a regulatory role in maintaining the integrity of the vascular wall. We speculate that dysregulated EphB4 function, caused either by structural changes in the protein, changes in the gene or protein expression or altered receptor signaling could result in intracranial vessel abnormalities that increase the risk of BAVM hemorrhage.

Our study had several limitations: (1) the analysis was restricted to whites, and results may not extend to other race/ethnic groups; (2) false-positive associations could be introduced by unrecognized population substructure differences between ICH and non-ICH cases; and (3) given the small size of the cohort, replication in additional cohorts is needed to provide a more reliable estimate of the effect size and rule out false-positive results. Future studies will need to evaluate a larger number of patients with BAVM, assess whether these EPHB4 SNPs also confer future ICH risk, and examine functionality.

In conclusion, we identified 2 SNPs located at the 5’ end of EPHB4, rs314313 and rs314308, that were associated with a reduced risk of hemorrhagic presentation in white patients with BAVM, but not with BAVM susceptibility. These findings suggest that genetic variation in EPHB4 contributes to the risk of hemorrhage in patients with BAVM and warrant further investigation into the role of Eph receptors in BAVM hemorrhage.

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Disclosures
None.

References
Clinical Perspective

Brain arteriovenous malformations (BAVMs) are a relatively rare but important cause of intracranial hemorrhage (ICH) in young adults, a potentially life-threatening disorder. Although the pathogenesis of BAVM and ICH is unknown, candidate genes that function in the development or maintenance of the vasculature may serve as markers of disease risk. The erythropoietin-producing hepatocellular receptor B4 (encoded by EPHB4) is involved in the development of the vasculature and functions in blood vessel permeability, inflammation, wound healing, and pathological angiogenesis. However, the role of EPHB4 gene polymorphisms as risk factors for BAVM and ICH has not yet been studied. In this genetic association study, we tested 8 single-nucleotide polymorphisms in the EPHB4 gene in 248 white patients with BAVM (93 ICH, 155 non-ICH) and 225 healthy controls for associations with BAVM and ICH at initial presentation. We found that 2 SNPs and haplotypes in EPHB4 were associated with a reduced risk of hemorrhagic presentation in white patients with BAVM but not with disease susceptibility. Our observations suggest that the risk of ICH presentation may differ between individuals with BAVM, depending on genetic risk factors that may affect the development and/or maintenance of the vasculature.
**EPHB4 Gene Polymorphisms and Risk of Intracranial Hemorrhage in Patients With Brain Arteriovenous Malformations**

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### SUPPLEMENTAL MATERIAL.

#### Supplemental Table. Clinical Characteristics of Phase I and Phase II Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BAVM Cases</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phase I n=146</td>
<td>Phase II n=102</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, mean ±SD, y</td>
<td>40.0 ±16.9</td>
<td>37.5 ±17.7</td>
<td>0.506†</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70 (47.9)</td>
<td>46 (45.1)</td>
<td>0.658</td>
</tr>
<tr>
<td>Female</td>
<td>76 (52.1)</td>
<td>56 (54.9)</td>
<td></td>
</tr>
<tr>
<td>ICH at initial presentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>56 (38.4)</td>
<td>37 (36.3)</td>
<td>0.739</td>
</tr>
<tr>
<td>No</td>
<td>90 (61.6)</td>
<td>65 (63.7)</td>
<td></td>
</tr>
<tr>
<td>BAVM size, mean size ±SD, cm*</td>
<td>2.9 ±1.6</td>
<td>2.9 ±1.5</td>
<td>0.961†</td>
</tr>
<tr>
<td>Deep venous drainage*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (19.1)</td>
<td>13 (17.1)</td>
<td>0.730</td>
</tr>
<tr>
<td>No</td>
<td>102 (80.9)</td>
<td>63 (82.9)</td>
<td></td>
</tr>
<tr>
<td>Recruitment site</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>UCSF</td>
<td>102 (69.9)</td>
<td>65 (63.7)</td>
<td>0.311</td>
</tr>
<tr>
<td>KPMCP</td>
<td>44 (30.1)</td>
<td>37 (36.3)</td>
<td></td>
</tr>
</tbody>
</table>

UCSF, University of California, San Francisco. KPMCP, Kaiser Permanente Medical Care Program of Northern California. Values are No. and (percent), unless indicated otherwise. * Counts do not add up to total due to missing data. A total of 180 patients had complete phenotypic data. P, χ² test, except for †t test.