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Authors
Weinsheimer, Shantel
Brettman, Ari D
Pawlikowska, Ludmila
et al.

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G Protein-Coupled Receptor 124 (*GPR124*) Gene Polymorphisms and Risk of Brain Arteriovenous Malformation

Shantel Weinsheimer[1], **Ari D. Brettman**[2], **Ludmila Pawlikowska**[1,3], **D. Christine Wu**[4], **Michael R. Mancuso**[4], **Frank Kuhnert**[4], **Michael T. Lawton**[5], **Stephen Sidney**[6], **Jonathan G. Zaroff**[6], **Charles E. McCulloch**[7], **William L. Young**[1,5,8], **Calvin Kuo**[4], and **Helen Kim**[1,7]

[1]Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco, San Francisco, CA, USA

[2]Department of Medicine, Harvard Medical School, and Cardiology Division, Massachusetts General Hospital, Boston, MA, USA

[3]Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA

[4]Department of Medicine, Hematology Division, Stanford University, Stanford, CA, USA

[5]Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA, USA

[6]Division of Research, Kaiser Permanente Medical Care Program of Northern California (KPNC), Oakland, CA, USA

[7]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA

[8]Department of Neurology, University of California, San Francisco, San Francisco, CA, USA

Abstract

Abnormal endothelial proliferation and angiogenesis may contribute to brain arteriovenous malformation (BAVM) formation. G protein-coupled receptor 124 (GPR124) mediates embryonic CNS angiogenesis; thus we investigated the association of single nucleotide polymorphisms (SNPs) and haplotypes in *GPR124* with risk of BAVM. Ten tagging SNPs spanning 39 kb of *GPR124* were genotyped in 195 Caucasian BAVM patients and 243 Caucasian controls. SNP and haplotype association with risk of BAVM was screened using χ^2 analysis. Associated variants were further evaluated using multivariable logistic regression, adjusting for age and sex. The minor alleles of 3 *GPR124* SNPs adjacent to exon 2 and localized to a 16 kb region of high linkage disequilibrium were associated with reduced risk of BAVM (rs7015566 A, P=0.001; rs7823249 T, P=0.014; rs12676965 C, P=0.007). SNP rs7015566 (intron 1) remained associated after permutation testing (additive model P=0.033). Haplotype analysis revealed a significant overall association (χ^2=12.55, 4 *df*, P=0.014); 2 haplotypes (ATCC, P=0.006 and GGCT, P=0.008) were associated with risk of BAVM. We genotyped a known synonymous SNP (rs16887051) in exon 2, however genotype frequency did not differ between cases and controls. Sequencing of conserved *GPR124* regions revealed a novel indel polymorphism in intron 2. Immunohistochemistry confirmed GPR124 expression in the endothelium with no qualitative difference in expression between BAVM cases and controls. SNP rs7015566 mapping to intron 1 of *GPR124* was associated with BAVM susceptibility among Caucasians. Future work is focused on investigating this gene region.

Correspondence to: Helen Kim, PhD, Department of Anesthesia and Perioperative Care, University of California, San Francisco, 1001 Potrero Avenue Box 1363, San Francisco, CA 94110, Tel: 415-206-4789; Fax: 415-206-8907, kimhel@anesthesia.ucsf.edu.

Keywords

Angiogenesis; Genetics; Intracerebral hemorrhage; Risk factor; Vascular malformation

Introduction

Brain arteriovenous malformations (BAVMs) are high-flow vascular lesions in which blood flows directly from the arterial to venous circulation with no intervening capillary bed. Patients with BAVM are susceptible to intracranial hemorrhage (ICH), and approximately half of all patients present with ICH [1–3]. The pathogenesis of BAVM is not yet defined, however both human and mouse studies support a role for dysregulated angiogenesis and inflammation [2, 4–10]. We recently identified polymorphisms in *ANGPTL4,* a pro- and anti-angiogenic mediator, that are associated with increased BAVM risk [11]. In addition, pro-inflammatory cytokines including interleukins IL-6 [12] IL-1β,[6] IL-1α,[13], IL-1RN [13], and transcription factors such as Notch 1 [14] and homeobox D3 (HOXD3) [15] can induce angiogenic activity that may contribute to BAVM. A highly positive correlation between angiopoietin-2 (ANG2) [16, 17] and vascular endothelial growth factor (VEGF) levels in BAVM surgical specimens suggests that angiogenic factors may contribute to vascular instability resulting in BAVM hemorrhage [18–20]. Genetic variants in *EPHB4*, a receptor important in arterio-venous differentiation and mediation of VEGF driven angiogenesis, were also associated with ICH susceptibility [5, 21].

The orphan G protein-coupled receptor GPR124/TEM5 has recently been described as a regulator of CNS-specific angiogenesis [22], which is upregulated in endothelial cells during tumor and physiologic angiogenesis [23, 24]. Complete or endothelial-specific inactivation of the GPR124 gene results in embryonic lethality by E15.5 due to CNS-specific angiogenesis arrest, while GPR124 overexpression leads to CNS-specific hyperproliferative vascular malformations that resemble venous angiomas [22]. Anderson *et al* subsequently reported that during development GPR124 is specifically expressed in the vasculature and is required for proper angiogenic sprouting into neural tissue [25]. Recent evidence also suggests GPR124 expression is required for CNS-specific vascularization and establishment of the blood-brain barrier [26]. These studies suggest a potential role for GPR124 in BAVM or ICH presentation. Thus, we hypothesized that polymorphisms in the *GPR124* gene may be associated with increased risk of BAVM susceptibility or with ICH presentation in BAVM cases.

Materials and Methods

Study Population

Our study included 195 Caucasian BAVM cases and 243 healthy Caucasian controls. BAVM cases were recruited at the University of California, San Francisco (UCSF) or Kaiser Permanente Medical Care Plan of Northern California (KPNC) as part of our larger UCSF-KPNC Brain AVM registry. Details on case identification, enrollment, ascertainment, verification of diagnosis, and data collection have been previously described [27–29] using standardized guidelines [30]. Controls were healthy volunteers with no significant medical history recruited from the same clinical catchment area for a pharmacogenetics study conducted at UCSF [31]. Informed consent was obtained on all study participants, and the study was approved by the Institutional Review Boards at UCSF and KPNC. The subset of patients who provided blood or saliva specimens, and self-reported as Caucasian, our largest ethnic subgroup, were eligible for this genetic study. The study population was restricted to Caucasians to reduce the potential for population stratification or confounding by race/ ethnicity.

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We also performed a secondary case-only analysis, comparing 81 ruptured with 114 unruptured BAVM cases at presentation. New intracranial blood on computed tomography or magnetic resonance imaging was used to define ICH presentation, and coded as 'ruptured' irrespective of clinical presentation. Cases without evidence of new bleeding and presenting with seizure, focal ischemic deficit, headache, apparently unrelated symptoms or asymptomatic were coded as 'unruptured'.

SNP Selection

Tagging SNPs in the GPR124 gene were selected from HapMap CEU population data (dbSNP build 126 on NCBI human genome build 36), using the Tagger algorithm [32] implemented in Haploview [33]. We used pairwise tagging to select a minimal set of tagSNPs with a minor allele frequency 5% such that all captured alleles are correlated at r^2 0.8 with a marker in that set. Ten SNPs capturing variation over a 39-kb region were selected for genotyping. Follow-up study included genotyping one synonymous SNP in exon 2 (rs16887051) in the same sample set.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a salt modification method (Gentra Systems, Minneapolis, MN, USA). Polymorphism-spanning fragments were amplified by polymerase chain reaction and genotyped by Beckman Coulter SNPstream 48plex technology. However, three SNPs (rs7015566, rs7813990, and rs12676965) failed multiplex assay and were genotyped as single assays using template-directed primer extension with fluorescence polarization detection (Acycloprime II; Perkin Elmer, Boston, MA, USA) [34]. For each SNP, all cases and controls were genotyped using the same method with 95% genotyping call rate in the combined set, and did not differ significantly between cases and controls.

Statistical Analysis

Demographic and clinical characteristics of the BAVM cases and healthy controls were compared using *t*-tests for continuous variables (presented as mean ± standard deviation) and χ^2 test for categorical variables.

Allelic Test of Association

Allele frequencies between BAVM cases and controls and between ruptured and unruptured cases were compared using χ^2 tests of association in PLINK version 1.06 [35]. To account for multiple comparisons, we performed 1000 permutations of case-control status, comparing each observed test statistic against the maximum of all permuted statistics over all ten SNPs. The empirical *P*-value thus controls the study-wide error rate. Our estimates suggest that with our study sample size we had >80% power to detect an OR 0.65 or 1.49 per risk allele if we assumed an allele frequency of 31% or more in controls. For SNP association with BAVM rupture, we had >80% power to detect an odds ratio of 0.38 or 2.02 per risk allele if we assumed an allele frequency of 16% or more in unruptured BAVM patients.

Genotypic Test of Association

Hardy-Weinberg equilibrium (HWE) was evaluated among controls using the χ^2 goodness-of-fit test implemented in Intercooled Stata software version 11 (StataCorp LP; College Station, TX) [36]. Genotypes were tested for association with BAVM using the χ^2 test (2 *df*). Since the true genetic model is not known, we also tested the recommended additive model (0, 1, or 2 copies of the minor allele) with permutation testing as described above to

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estimate the odds ratios (OR) and 95% confidence interval (CI), adjusting for age and sex. Multivariable logistic regression analysis was performed using PLINK version 1.06 [37].

Haplotype Test of Association

Four-SNP fixed window haplotype frequencies were inferred from unphased genotype data using the expectation-maximization (EM) algorithm. Both a global likelihood ratio test of association comparing the overall haplotype distribution between cases and controls with degrees of freedom (*df*) equal to number of haplotypes tested −1, and haplotype-specific tests of association comparing each haplotype versus all other haplotypes (i.e., 1 *df*) were performed using PLINK version 1.06.[35] Only common haplotypes with a minor haplotype frequency (MHF) 1% were considered for analysis and significance was set at $\alpha<0.05$.

Sequencing of GPR124 Exon 2 and Conserved Gene Regions

To identify new variants in associated regions, we sequenced *GPR124* exon 2 and four highly conserved regions within the surrounding 16 kb linkage disequilibrium block in 24 BAVM cases. The human genome reference sequence (hg18) was used as the reference. Sequencing was successful in 20 cases. PCR and sequencing primers were designed using Primer3 [38]. Primer sequences and PCR reaction conditions are available upon request. PCR was cleaned up with 1× SAP PCR Clean-Up Reagent (Perkin-Elmer Life Sciences Inc., Waltham, MA, USA). Sequencing was performed in one direction using BigDye Terminator v3.1 (ABI; Foster City, CA, USA). Excess dye terminators were removed using genCLEAN (Genetix, New Milton, Hampshire, UK) plates following manufacturer's instructions before automated capillary sequencing on an ABI3730 DNA Analyzer. Sequences were visualized in Sequencher (Gene Codes Corp., Ann Arbor, MI, USA).

Immunohistochemistry

Adult human tissues from normal brain, hereditary hemorrhagic telangiectasia (HHT), and pilocytic astrocytoma were fixed in 4% paraformaldehyde (PFA) for one hour, cryoprotected in 30% sucrose and embedded in OCT. Frozen sections (10 μm) were stained for GPR124 and CD31 expression as previously described [22] using rabbit anti-GPR124 and hamster anti-CD31 (Millipore). Fluorescein conjugated isolectin B4 (Vectorlabs) was added to the secondary antibody mix to mark endothelial cells.

Using single-label immunohistochemistry, we evaluated GPR124 expression in unruptured BAVM vessels from 2 patients with sporadic BAVM and 3 control brain samples including one superficial temporal artery (STA) and two cerebral cortex samples obtained by temporal lobectomy for medically intractable seizure. Immunostaining details are located in the Electronic Supplementary Material.

Results

The demographic and morphological characteristics for the patients with BAVM and controls are summarized in Table 1. Controls were significantly younger than BAVM cases ($P<0.001$); there was no significant difference in sex. Among cases, 42% presented with hemorrhage, 36% had deep venous drainage, and mean BAVM size was 2.8 ± 1.4 cm.

Association of *GPR124* SNPs with BAVM

We genotyped 10 tagging SNPs located in the *GPR124* gene (Table 2) in 195 Caucasian BAVM cases and 243 healthy Caucasian controls. All SNPs were polymorphic (minor allele frequency >1%), and in Hardy-Weinberg equilibrium among the controls ($P > 0.05$). Minor allele frequencies were similar between study controls and HapMap CEU population.

Genotype frequencies (Table 3) differed significantly between BAVM and controls for rs7015566 (P=0.007), rs7823249 (P=0.049), and rs12676965 (P=0.023).

Allelic and additive model association analysis identified 3 markers associated with reduced risk of BAVM after adjusting for age and sex (Table 4, additive model: rs7015566, P=0.003; rs7823249, P=0.012; rs12676965, P=0.024). SNP rs7015566 located in intron 1 remained associated after permutation testing (P=0.033).

These polymorphisms localize to a 16 kb region of high LD, suggesting they may serve as markers for functional SNPs involved in BAVM pathogenesis (Figure 1). Haplotype analysis of SNPs within this LD block (n=4) revealed a significant overall association (Table 5, χ^2=12.55, 4 *df*, P=0.014); 2 haplotypes (ATCC, P=0.006 and GGCT, P=0.008) were associated with risk of BAVM. The most significantly associated haplotype (ATCC) was consistent with the individual SNP analysis, as it contains the minor allele for the significantly associated SNP (rs7015566, A). In a follow-up analysis, we also genotyped synonymous SNP rs16887051 in exon 2, which was not associated with BAVM susceptibility (P=0.744).

In a secondary analysis, we assessed whether any of the GPR124 SNPs were associated with ICH presentation among BAVM patients. 81 of 195 (42%) BAVM cases presented with ICH. SNP rs17433803 was marginally associated with ICH presentation after adjustment for age and sex (OR=1.95, 95% CI=1.15 – 3.30, unadjusted P=0.013); however, this SNP was not associated after permutation testing (P=0.10). Other *GPR124* SNPs (P>0.05, data not shown) nor haplotypes (χ^2=7.42, 4 *df*, *P*=0.115) were associated with the risk of ICH presentation.

Sequencing

Sequencing of exon 2 and four highly conserved regions within the surrounding LD block in 24 BAVM cases revealed a novel complex insertion deletion polymorphism (indel) located in intron 2 (hg18, chr8:37799084 – 37799104) (Figure 2). Compared to reference sequence (hg18), 3/20 individuals were homozygous for the insertion. This indel polymorphism has not been previously reported, however there are 8 known SNPs that overlap it (dbSNP 135). This indel appears to be linked to the BAVM associated rs7015566 because 2/20 BAVM cases with the AA genotype are also homozygous for the insertion sequence in the conserved region (CONS4), one BAVM case that was heterozygous for SNP rs7015566 is also homozygous for the indel and none of the rs7015566 GG samples bear the indel.

GPR124 Expression in Human Vasculature

Protein expression analysis showed that GPR124 is co-expressed with CD31 in both normal human brain vasculature and two pathological brain samples including BAVM in a patient with HHT and a pilocytic astrocytoma sample (Figure 3A). We also evaluated GPR124 protein expression in unruptured BAVM vessels from patients with sporadic BAVM compared to control brain vessels (STA and cerebral cortex). GPR124 protein had apparent localization to the endothelium in both BAVM and control vessels, shown by the intense brown GPR124 staining along the endothelial cell layer (Figure 3B inset, black arrows). We did not observe any qualitative difference in GPR124 protein expression between BAVM and control vessels.

Discussion

We provide the first report of an association between genetic variation in the *GPR124* gene with risk of BAVM. In this population of Caucasians, we identified SNP rs7015566 located in intron 1 that contributes to a reduced risk of BAVM in individuals who carry the minor

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allele. Case-only analyses suggest this finding is specific to BAVM susceptibility; however, our study sample size was too small to detect significant differences in MAF associated with ICH presentation. Haplotypes were consistent with the SNP analysis; the minor allele A for rs7015566 was present in the most significant haplotype (ATCC), which was associated with reduced BAVM risk. The associated SNP was selected as a haplotype-tagging SNP and is located in a well-conserved region with no known function. Hence, the SNP is likely not causal, but a surrogate marker in LD with functional polymorphisms located elsewhere in the *GPR124* gene, in a closely neighboring gene or in a regulatory element.

GPR124 encodes the GPR124 protein that belongs to the large family of long N-terminal group B (LNB) G protein-coupled receptors (GPCRs). GPR124, originally named tumor endothelial marker 5 (TEM5), is an orphan receptor whose signaling mechanism is not yet known. This gene has 4 known protein-coding transcripts and 2 noncoding transcripts. However, SNP rs7015566 and the novel indel polymorphism identified in this study, are not located near a splice site (closest site is 1274 bp downstream from rs7813990); therefore, these polymorphisms are not likely to influence splicing efficiency. The associated SNP is located near exon 2, which encodes one of the four leucine-rich repeat protein domains that may provide a framework for GPR124 protein-protein interactions and facilitate involvement in a variety of biological processes such as angiogenesis and vasculogenesis. It has been well-documented that noncoding sequences may function as gene regulatory elements [39]. Thus, one explanation for our findings is that SNP rs7015566 may be in disequilibrium with other *GPR124* SNPs located in exons or regulatory elements that may be protective of BAVM by affecting GPR124 gene or encoded protein expression, or influencing receptor-ligand binding.

We recently proposed a “response-to-injury” paradigm to explain sporadic BAVM pathogenesis [10]. In this model, an inciting event (e.g., trauma, infection, inflammation, etc.) that normally triggers angiogenesis, endothelial mitogenesis, and vascular stabilization, instead shifts toward an abnormal dysplastic response when there is an underlying genetic background or environmental insult. While the exact mechanism is unknown, the high prevalence of BAVM in patients with HHT (mutations in *ACVRL1, ENG,* or *SMAD4*) suggests genetic variation in TGF-β signaling genes or angiogenic factors, such as *GPR124*, may contribute to an underlying genetic background that influences sporadic AVM pathogenesis. In our study, we observed expression of GPR124 protein in BAVM vessels from both sporadic and HHT patients. Consistent with previous studies, GPR124 localized to the endothelium [40] and expression levels qualitatively appeared similar to control vessels. Brain AVMs from HHT patients may have differences in GPR124 expression and/or localization compared to sporadic brain AVMs; however, we were not able to identify such differences in the current study. Our immunohistochemical analysis is a descriptive study of GPR124 expression in a limited number of AVM and control brain vessels. A larger number of patient samples would need to be evaluated to accurately estimate any difference in GPR124 expression between AVM and controls. While we did not detect a difference in GPR124 expression in AVMs and controls, it is possible that GPR124 expression may be altered during development or at different stages of brain AVM pathogenesis (i.e., AVM formation or growth). While these data support a role for GPR124 in the adult brain vasculature, additional studies will be needed to determine the functional role of GPR124 genetic variation in human AVM pathogenesis.

Recent evidence described by Anderson *et al* (2011) suggests that GPR124 normally modulates signaling through the TGF-β pathway [25]. Further, deletion of GPR124 resulted in angiogenic defect [22] and increased expression of endoglin, a coreceptor for Alk1 [25]. *In vitro* studies have also suggested a potential functional role for GPR124 in endothelial cell migration and proliferation [22, 41]. In addition, a soluble fragment (sGPR124) is shed

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by endothelial cells during capillary-like network formation and upon growth factor stimulation [40]. Interestingly, proteolytically processed sGPR124 mediates endothelial cell survival during angiogenesis by linking integrin αVβ3 to glycosaminoglycans [40]. Taken together with our current genetic association finding, it is plausible that GPR124 may be involved dysregulated angiogenesis or endothelial cell function that influences the development of sporadic BAVMs. Thus, a better understanding of the relationship between GPR124 and TGF-β signaling may offer insight into BAVM pathogenesis.

Our study had several limitations: (1) the analysis was restricted to Caucasians, and risk estimates may differ or be absent in other race/ethnic groups; (2) individuals were self-reported Caucasian therefore population stratification may exist; (3) replication in additional cohorts is needed to provide a more reliable estimate of the effect size and rule out false-positive results; and (4) limited tissue was available for patients homozygous for rs7015566 major allele, thus we were unable to estimate the correlation between rs7015566 genotype on GPR124 protein expression. Future studies will need to evaluate a larger number of patients with BAVM to examine functionality.

Conclusions

In conclusion, SNP rs7015566 located in intron 1 of *GPR124* was associated with reduced risk of BAVM in Caucasian patients. These findings suggest that genetic variation in *GPR124* contributes to BAVM risk and warrant further investigation into the role of GPCRs in BAVM pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ACVRL1	activin A receptor type II-like 1
ANG2	Angiopoietin 2
ANGPTL4	Angiopoietin-like 4
BAVM	brain arteriovenous malformation
CI	confidence interval
CNS	central nervous system
df	degrees of freedom
ENG	endoglin
EPHB4	EPH receptor B4

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GPCR	G protein-coupled receptor
GPR124	G protein-coupled receptor 124
HHT	hereditary hemorrhagic telangiectasia
HOXD3	Homeobox D3
HWE	Hardy-Weinberg equilibrium
ICH	intracranial hemorrhage
IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-1RN	Interleukin 1 receptor antagonist
IL-6	Interleukin 6
LD	linkage disequilibrium
LNB	long N-terminal group B
MAF	minor allele frequency
MHF	minor haplotype frequency
OCT	optimized cutting temperature
OR	Odds ratio
PCR	polymerase chain reaction
PFA	paraformaldehyde
SMAD4	SMAD family member 4
SNP	single nucleotide polymorphism
STA	superficial temporal artery
TEM5	Terminal endothelial marker 5
TGF-β	Transforming growth factor beta
VEGF	Vascular endothelial growth factor

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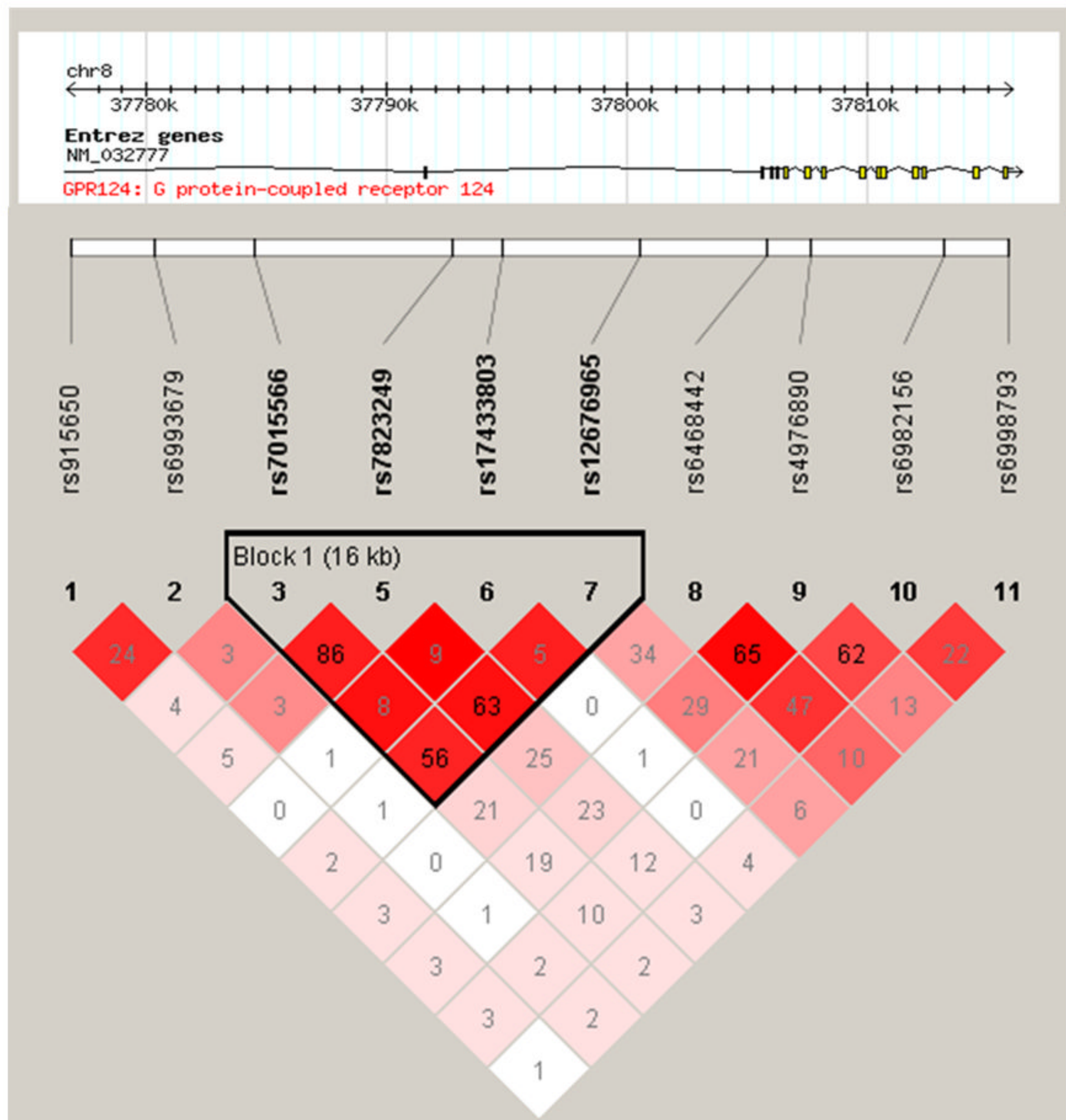

Figure 1.

Linkage disequilibrium (LD) structure for *GPR124* locus. *GPR124* SNPs are represented in order on the chromosome. LD between SNPs is represented both numerically (r^2) and by the depth of shading (D) computed using all genotype data from the 195 BAVM patients and 243 controls. One haplotype block exists in this *GPR124* region spanning SNPs rs7015566 – rs12676965.

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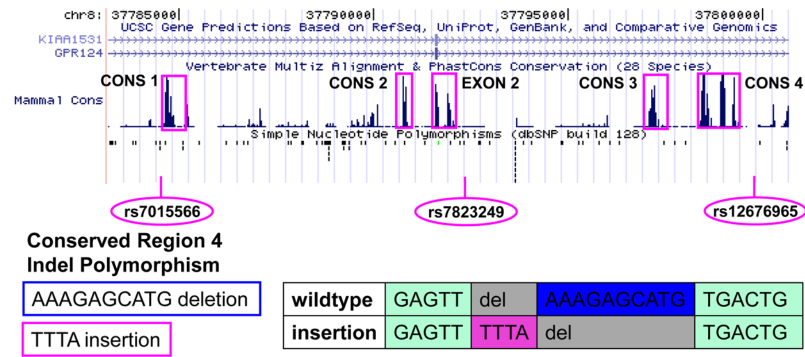

Figure 2.

Identification of a complex insertion/deletion polymorphism in *GPR124* gene conserved region. Sequencing of exon 2 and 4 conserved intronic regions across *GPR124* led to the identification of a 4 base insertion (TTTA, pink box) and 10 base deletion (blue box) located in conserved region 4 (CONS4), in a region between two highly conserved sequences (hg18, chr8:37799084 – 37799104).

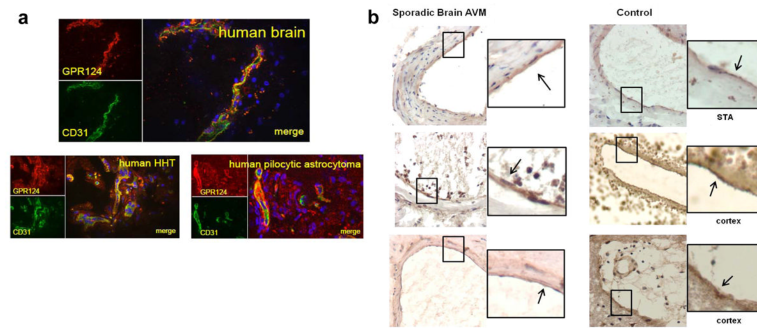

Figure 3.
GPR124 protein expression in human brain vessels. **a** Immunofluorescence shows co-expression (merge, yellow) of GPR124 (red) and CD31 endothelial cell marker (green) in normal (top panel) and malformed human vessels (BAVM from HHT patient and human pilocytic astrocytoma). **b** GPR124 immunostaining in sporadic BAVM vessels (left panel) and control brain vessels (right panel) including superficial temporal artery (STA) and cerebral cortex vessels. GPR124 protein has apparent localization to the endothelium (black arrows) in both AVM and control brain vessels.

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Table 1

Demographic and Clinical Characteristics of Study Cohort

Characteristics	BAVM Cases		Controls		P^*
	No.	%	No.	%	
Total participants (n=438)	195	45	243	55	
Age, years (mean ± SD)	38.0 ± 16.3		30.6 ± 5.8		<0.001
Female Sex	114	59	138	57	0.801
Hemorrhagic presentation	81	42	n/a	n/a	n/a
BAVM size, cm (mean ± SD)	2.8 ± 1.4	n/a	n/a	n/a	n/a
Venous Drainage					
Superficial only	92	59	n/a	n/a	n/a
Any deep	56	36	n/a	n/a	n/a

* *t*-test for continuous variables and χ^2 for categorical variables.

n/a = not applicable

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Table 2

GPR124 Polymorphisms Selected for Genotyping

Location	dbSNP ID	Base Change
Intron 1	rs915650[a]	C>T
Intron 1	rs6993679[a]	G>A
Intron 1	rs7015566[b]	G>A
Exon 2	rs16887051[a]	G>T
Intron 2	rs7823249[a]	C>A
Intron 2	rs17433803[a]	T>C
Intron 2	rs12676965[b]	G>A
Intron 3	rs6468442[a]	G>T
Intron 7	rs4976890[a]	G>A
Intron 13	rs6982156[a]	C>T
Intron 15	rs6998793[a]	G>A

[a]SNP genotyped using SNPstream 48plex technology

[b]SNP genotyped using fluorescence-polarization detection using template-directed dye-terminator incorporation assay (FP-TDI)

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Table 3

Genotype and Allele Frequencies of *GPR124* Polymorphisms in BAVM Cases and Controls

SNP Genotype	Case, n (%)	Controls, n (%)	P^*
rs915650			
CC	75 (38.5)	86 (35.5)	0.351
CT	102 (52.3)	123 (50.8)	
TT	18 (9.2)	33 (13.6)	
C	(64.6)	(60.9)	0.266
T	(35.4)	(39.1)	
rs6993679			
AA	4 (2.1)	6 (2.5)	0.968
AG	49 (26.1)	62 (25.7)	
GG	135 (71.8)	173 (71.8)	
A	(15.2)	(15.4)	0.938
G	(84.8)	(84.6)	
rs7015566			
AA	12 (6.2)	28 (11.5)	0.007
AG	58 (29.9)	95 (39.1)	
GG	124 (63.9)	120 (49.4)	
A	(21.1)	(31.1)	0.001
G	(78.9)	(68.9)	
rs16887051			
AA	156 (90.7)	221 (92.5)	0.520
AG	16 (9.3)	18 (7.5)	
GG	0 (0)	0 (0)	
A	(95.3)	(96.2)	0.817
G	(4.7)	(3.8)	
rs7823249			
GG	116 (61.3)	121 (51.5)	0.049
GT	64 (33.9)	91 (38.7)	
TT	9 (4.8)	23 (9.8)	
G	(78.3)	(70.8)	0.014
T	(21.7)	(29.2)	
rs17433803			
AA	9 (4.7)	12 (4.9)	0.991
AC	60 (31.1)	75 (30.9)	
CC	124 (64.3)	156 (64.2)	
A	(20.2)	(20.4)	0.953
C	(79.8)	(79.6)	
rs12676965			
CC	4 (2.1)	17 (7.1)	0.023
CT	50 (26.5)	75 (31.1)	

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SNP Genotype	Case, n (%)	Controls, n (%)	*P* *
TT	135 (71.4)	149 (61.8)	
C	(15.3)	(22.6)	0.007
T	(84.7)	(77.4)	
rs6468442			
AA	4 (2.1)	13 (5.4)	0.183
AG	68 (35.1)	88 (36.2)	
GG	122 (62.9)	142 (58.4)	
A	(19.6)	(23.5)	0.168
G	(80.4)	(76.5)	
rs4976890			
GG	99 (51.3)	113 (46.9)	0.552
GT	82 (42.5)	108 (44.8)	
TT	12 (6.2)	20 (8.3)	
G	(72.5)	(69.3)	0.297
T	(27.5)	(30.7)	
rs6982156			
AA	92 (47.2)	109 (44.9)	0.629
AG	88 (45.1)	109 (44.9)	
GG	15 (7.7)	25 (10.2)	
A	(69.7)	(67.3)	0.437
G	(30.3)	(32.7)	
rs6998793			
CC	73 (37.6)	93 (38.8)	0.892
CT	87 (44.9)	109 (45.4)	
TT	34 (17.5)	38 (15.8)	
C	(60.0)	(61.5)	0.673
T	(40.0)	(38.5)	

*P-values calculated from χ^2 test (2 degrees of freedom); genotypic and allelic models.

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Table 4

Association of *GPR124* Polymorphisms with BAVM

	Additive Model[a]			
Polymorphism	**OR**	**95% CI**	***P***	P_{perm}[b]
rs915650_T	0.82	0.61 to 1.12	0.215	0.813
rs6993679_A	1.05	0.70 to 1.57	0.809	1.000
rs7015566_A	0.62	0.45 to 0.85	0.003	0.033
rs7823249_T	0.66	0.47 to 0.91	0.012	0.099
rs17433803_A	0.95	0.68 to 1.35	0.792	1.000
rs12676965_C	0.65	0.45 to 0.94	0.021	0.149
rs6468442_A	0.77	0.54 to 1.09	0.141	0.644
rs4976890_T	0.85	0.62 to 1.18	0.328	0.944
rs6982156_G	0.89	0.65 to 1.22	0.482	0.994
rs6998793_T	1.13	0.85 to 1.50	0.413	0.981

OR, odds ratio; CI, confidence interval.

[a]Additive model includes adjustment for age and sex.

[b]*P*-value from permutation testing.

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Table 5

Association of *GPR124* Haplotypes with Risk of BAVM

Haplotype[a]	Frequency Estimates		χ^2	*df*	*P*
	Cases	Controls			
Global	**NA**	**NA**	**12.55**	**4**	**0.014**
ATCC	**0.14**	**0.22**	**7.67**	**1**	**0.006**
GGAT	0.22	0.20	0.20	1	0.653
ATCT	0.05	0.07	1.65	1	0.199
AGCT	0.01	0.02	1.26	1	0.261
GGCT	**0.58**	**0.49**	**7.14**	**1**	**0.008**

[a]Fixed window haplotype, 5′ →3′ rs7015566, rs7823249, rs17433803, rs12676965 *df*, degrees of freedom