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Common variants on 9p21.3 are associated with brain arteriovenous malformations with accompanying arterial aneurysms

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Abstract

Objective—To investigate whether previously reported 9p21.3 single nucleotide polymorphisms (SNPs) are associated with risk of brain arteriovenous malformations (BAVMs), which often have accompanying arterial aneurysms. Common variants in the 9p21.3 locus have been reported to be associated with multiple cardiovascular phenotypes, including coronary artery disease and intracranial aneurysms (rs10757278 and rs1333040).

Methods—We used data from 338 BAVM cases participating in the University of California, San Francisco-Kaiser Brain AVM Study Project and 504 healthy controls to evaluate genotypes for seven common SNPs (minor allele frequency>0.05) that were imputed using 1000 Genomes Phase 1 European data (R^2>0.87). Association with BAVM was tested using logistic regression adjusting for age, sex and the top three principal components of ancestry. Subgroup analysis

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Contributors

Planned and performed statistical analysis and drafted the manuscript: NB. Designed the study, supervised data collection and analysis, and edited the manuscript: HK. Performed or supervised laboratory and genetic analyses and edited the manuscript: LP. Performed additional statistical analysis with supervision and guidance from CEM and MS: JN, SW and NB. Collected data from BAVM cases and assisted with overall study design: JGZ, SS (Kaiser), and WLY (UCSF).

Competing interests

None.

included 205 BAVM cases with aneurysm data: (a) 74 BAVM with aneurysm vs. 504 controls, and (b) 131 BAVM without aneurysm vs. 504 controls.

Results—We observed suggestive association with BAVM and rs10757278-G (OR=1.23, 95% CI=0.99–1.53, P=0.064) and rs1333040-T (OR=1.27, 95% CI=1.01–1.58, P=0.04). For rs10757278-G, the association was stronger in BAVM cases with aneurysm (OR=1.52, 95% CI=1.03–2.22, P=0.032) than in BAVM without aneurysm (OR=0.98, 95% CI=0.72–1.34, P=0.91). Similar patterns of effects were observed for rs1333040 and for other SNPs in linkage disequilibrium (r^2>0.8) with rs10757278.

Conclusions—Common 9p21.3 variants showed similar effect sizes for association with BAVM as previously reported for aneurysmal disease. The association with BAVM appears to be explained by known associations with aneurysms, suggesting that BAVM associated aneurysms share similar vascular pathology mechanisms with other aneurysm types.

Keywords

cerebral arteriovenous malformations; intracranial aneurysms; genetic association studies

INTRODUCTION

The 9p21.3 locus is one of the strongest and most robustly replicated genetic loci identified by genome-wide association studies (GWAS) of cardiovascular diseases, including coronary artery disease (CAD), myocardial infarction (MI), carotid plaque, stroke, peripheral artery disease, intracranial aneurysms (IA), and abdominal aortic aneurysms (AAA).[1] The CAD-associated haplotype spans approximately 50 kb from rs12555547 to rs1333050, and contains protein-coding genes *CDKN2A* and *CDKN2B* in addition to a non-coding RNA (*CDKN2B-AS1*, HGNC: 34341), whose expression has been shown to be regulated by genetic variants in the 9p21 locus.[2] Interestingly, associations with this locus are independent of known cardiovascular risk factors such as hypertension and hyperlipidemia, [1] suggesting that variants confer risk through a different mechanism than traditional cardiovascular risk factors. In particular, SNPs rs10757278 [1] and rs1333040 [3] have been associated with IA in several studies, and rs1333040 has recently been reported to be associated with brain arteriovenous malformations (BAVMs) in an Italian cohort.[3]

BAVMs are a rare but leading cause of haemorrhagic stroke in children and young adults, leading to persistent morbidity and even mortality.[4] The AVM nidus is a tangle of poorly formed blood vessels, with direct shunting of blood from the arterial to the venous circulation without an intervening capillary bed and usually accompanied by high flow rates. The pathogenesis of BAVM remains unknown but risk factors for rupture are different from aneurysmal subarachnoid haemorrhage.[4] However, BAVMs often have coexisting arterial aneurysms located in the feeding arteries supplying the shunt flow or within the AVM nidus. The purpose of this study was to investigate whether common genetic variants in the CAD haplotype of the 9p21.3 locus are associated with BAVM and whether this association is explained by the presence of associated aneurysms.

METHODS

Study population

We used existing genome-wide association data from 371 BAVM cases participating in the UCSF-Kaiser Brain AVM Study Project [5] and 563 healthy controls.[6, 7] All subjects self-reported as Caucasian. Written informed consents were obtained from all subjects and the study was approved by the respective Institutional Review Boards.

BAVM diagnosis, morphological, and clinical characteristics were recorded using standardized definitions.[8] The diagnosis of AVM is based on evidence of shunting on digital subtraction angiography. AVM morphological characteristics were recorded from magnetic resonance imaging (MRI) and angiography by an interventional neuroradiologist.[9] Aneurysms were defined as saccular luminal dilatations of the arteries detected on angiography, and were further classified into flow-related aneurysms, intranidal aneurysms, and aneurysms unrelated to the shunt flow to the BAVM.[9] We defined “associated aneurysms” as aneurysms present in feeding arteries supplying the shunt flow (flow-related) or within the nidus (intranidal).

SNP genotyping, imputation and quality control

Subjects provided blood or saliva specimens for DNA extraction. Cases and controls were genotyped in the same laboratory using the Affymetrix® Genome-Wide Human SNP Array 6.0, and genotypes were called using Birdseed (version 2). Samples with genotyping call rate <95%, sex mismatches, or cryptic duplicates were excluded. SNPs that deviated significantly from Hardy-Weinberg equilibrium ($P<10^{-5}$) in the controls and with call rates <95% were also removed, yielding 338 cases and 504 controls for analysis.

We selected seven SNPs in the 9p21.3 CAD haplotype (rs1537378, rs10757274, rs2383206, rs1333049, rs2383207, rs10757278 and rs1333040) based on previous studies;[1, 3] associated phenotypes are shown above each SNP in Figure 1. Since six of the seven SNPs were not covered on the SNP array, we performed imputation across the genome with the 1000 Genomes Project European haplotypes as a reference using MaCH [10] and Minimac.[11] All seven SNPs were well-imputed ($R^2>0.87$) with minor allele frequency (MAF)>1%. Pairwise linkage disequilibrium (LD) between SNPs was assessed using r^2, with values >0.8 indicating high LD (Figure 1). For technical validation, we genotyped rs10757278 using TaqMan® assay C_11841860_10 (Applied Biosystems) in BAVM cases. Access to control DNA was unavailable.

Statistical analysis

To adjust for population stratification, we performed principal components analysis on 72,456 unlinked SNPs ($r^2<0.2$) with MAF>0.05 using EIGENSTRAT.[12] Association of the imputed genotype dosages of 7 SNPs with BAVM was tested by logistic regression analysis assuming an additive genetic model and adjusting for age, gender and the top three principal components using MaCH2dat software (http://www.sph.umich.edu/csg/abecasis/mach/download). Statistical significance was defined as p<0.017 after correcting for three SNPs (SNPs 3–7 are in strong LD, $r^2>0.8$, Figure 1) using the Bonferroni procedure. We

performed two sub-group analyses in the 205 cases with aneurysm data: (1) 74 BAVM with aneurysms vs. 504 controls; and (2) 131 BAVM without aneurysms vs. 504 controls.

RESULTS

There was no significant gender difference between cases and controls (P=0.22). However, controls were significantly older than cases (49 ± 14 years vs. 39 ± 18 years, respectively, P<0.001). About 36% of BAVM cases had associated flow-related or intranidal aneurysms, 3% had aneurysms unrelated to the shunt flow to the BAVM, and 62% had no aneurysms. BAVM cases with associated aneurysms were older (P=0.07), had higher Spetzler-Martin grade (P=0.027), and larger AVM size (P<0.001) compared to BAVM cases without associated aneurysms (Supplemental Table).

All seven SNPs showed a trend toward association with BAVM with an average effect size of 1.24 (Table 1); none were significantly associated with BAVM after correction for multiple testing. The overall association with BAVM was 1.27 for rs1333040-T (P=0.04) and 1.23 for rs10757278-G (P=0.064). When restricting the analysis to 74 BAVM with associated aneurysms, we observed a stronger effect size for rs1333040-T, rs10757278-G, and for all SNPs in LD with rs10757278-G (Table 1 and Figure 1); only rs1537378 did not follow a similar pattern. In contrast, no association was observed in 131 BAVM without associated aneurysms for any SNPs (Table 1). Technical validation of rs10757278 in BAVM cases yielded greater than 96% concordance with genotypes predicted from imputation.

Expanding the search to all 79 well-imputed SNPs in the 9p21.3 CAD haplotype did not identify any other SNPs significantly associated with BAVM after correction for multiple testing using the Bonferroni procedure (data not shown). Sub-group analysis revealed two SNPs with stronger association and larger effect size in BAVM with associated aneurysms: rs10217586-T (OR=1.73, 95% CI=1.16–2.58, P= 0.006) and rs1333045-C (OR=1.72, 95% CI=1.16–2.57, P=0.006). No association was observed between these SNPs and BAVM without associated aneurysms (P>0.77). These 2 SNPs are in strong LD (r^2> 0.95) with each other and in strong LD with rs10757278 (r^2 > 0.8).

DISCUSSION

In this study, we observed suggestive associations of seven common 9p21 SNPs with BAVM. Overall, the effect size and significance level of the association were stronger when restricting analysis to BAVM with associated aneurysms for SNPs in LD with rs10757278 in addition to rs1333040, both SNPs previously reported to be associated with IA. No association was observed between 9p21 SNPs and BAVM without associated aneurysms, suggesting that the association with BAVM is driven by the presence of aneurysms. The effect size for rs10757278 (OR=1.23) was similar to that found in previous studies of aneurysmal diseases.[1] Recently, Sturiale et al [3] reported an association of rs1333040-T in an Italian cohort of 73 BAVM cases and 103 controls with a higher effect size compared to our cohort (OR=1.65, P=0.01). Our results replicate this association in an independent cohort, and further suggest that the association with BAVM at the 9p21 locus is driven by the presence of associated aneurysms.

Examination of the remaining imputed SNPs within the 9p21.3 CAD haplotype revealed no significant associations with BAVM after correction for multiple testing. However, a similar pattern was observed with suggestive association of two SNPs (rs10217586-T and rs1333045-T) in strong LD with rs10757278 among BAVMs with aneurysms (P=0.006) and not among BAVMs without aneurysms (P>0.77). Both rs1333045 and rs10757278 have also been previously reported to be associated with aneurysmal subarachnoid haemorrhage in a Swedish population.[13]

Our finding that the same 9p21 SNPs are also associated with increased risk of BAVM with coexisting arterial aneurysms suggests that BAVM associated aneurysms share similar vascular pathology mechanisms with other aneurysmal diseases. Since both aneurysms of feeding arteries supplying BAVMs and intracranial aneurysms in general are both subject to high-flow arterial conditions (though higher flows should be present in arteries supplying AVMs as compared to arteries not supplying AVMs), genetic variation alone may not be sufficient for aneurysm formation. Alternatively, high-flow conditions may not be sufficient for aneurysm formation either, as feeding artery aneurysms are not observed in young vein of Galen malformation patients who have very high flow rates (personal communication, SWH) and are rarely seen in children with BAVMs,[14] suggesting that multiple factors contribute to BAVM associated aneurysms, including time for formation and growth.

The prevalence of aneurysms associated with BAVM varies widely depending on the definition of aneurysm, type of angiography used (selective vs. superselective), and referral patterns.[15] In our study, 36% of BAVM patients had associated aneurysms, which is higher than that reported in some referral cohorts [15, 16] but lower than that reported in a large series from Germany (46%)[17] and a smaller series undergoing superselective angiography (58%).[18] There may be selection bias of cases included in this genetic association study, although the aneurysm prevalence is very similar to an earlier study by our group that did not select BAVM patients based on DNA availability (34%).[9]

In conclusion, these results suggest that the observed association of 9p21 SNPs with BAVM is likely explained by the presence of coexisting aneurysms, and that BAVM-associated aneurysms may share similar vascular pathology mechanisms as other aneurysmal diseases. Our results do not have immediate clinical implications; however, they broaden our understanding of possible genetic mechanisms underlying aneurysm formation across vascular diseases that may be used to identify future candidate targets for therapy or help develop vessel-wall imaging for feeding artery aneurysms that may help better risk-stratify patients. Thus, our findings should be viewed as hypothesis-generating requiring replication in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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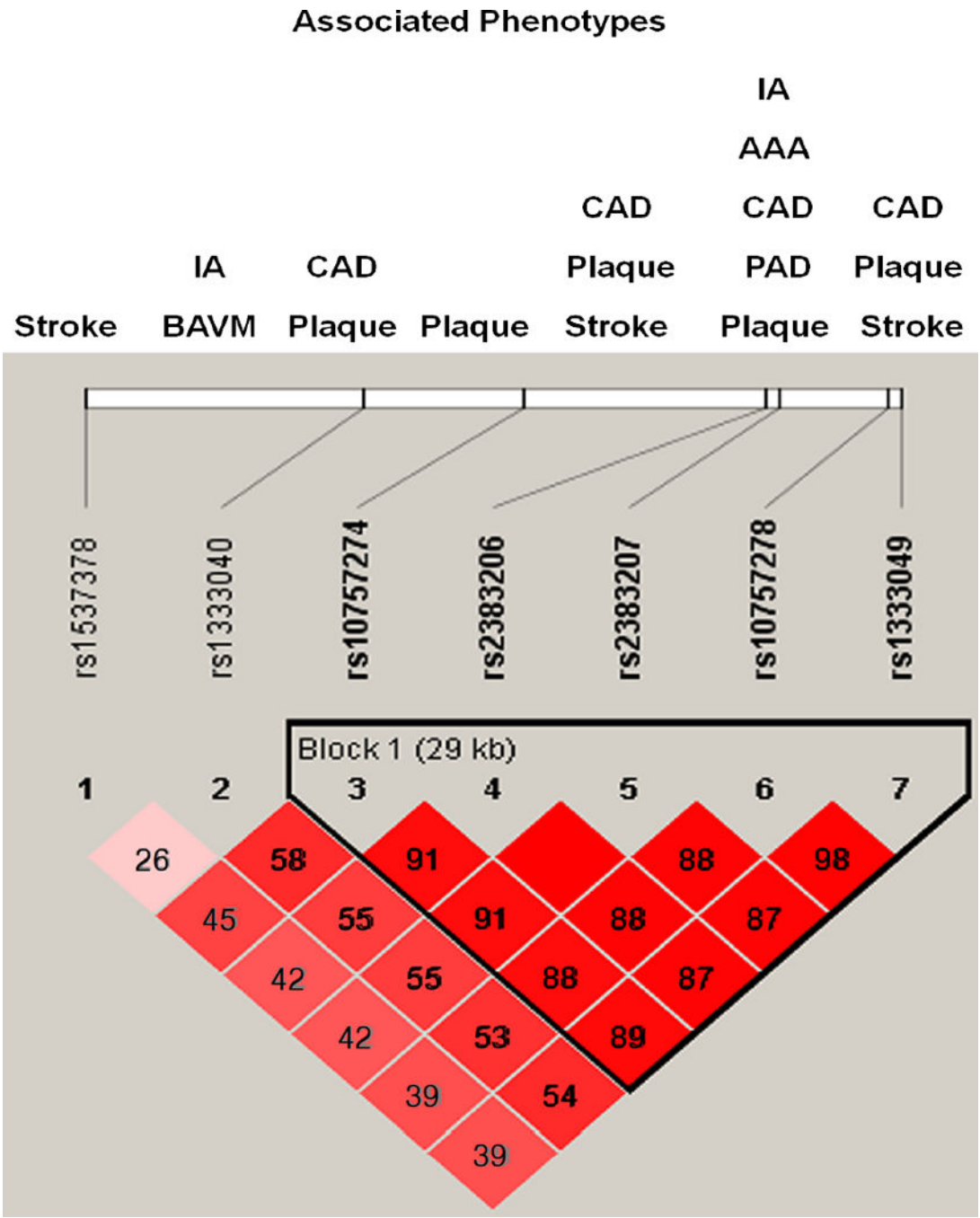

Figure 1.

Table 1

Association of 7 chr9p21 SNPs with BAVM, BAVM with associated aneurysms, and BAVM without associated aneurysms.

				* 338 BAVM vs. 504 Controls		74 BAVM+aneurysm vs. 504 Controls		131 BAVM-aneurysm vs. 504 Controls	
Associated Phenotype	**Variant**	**Allele**	**Freq**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
Stroke	rs1537378	C	0.62	1.24 (1.00–1.54)	0.05	1.21 (0.83–1.77)	0.31	1.10 (0.82–1.49)	0.52
BAVM, IA	rs1333040	T	0.58	1.27 (1.01–1.58)	0.04	1.45 (0.98–2.15)	0.06	1.02 (0.75–1.40)	0.89
CAD, Plaque	rs10757274	G	0.50	1.25 (1.01–1.54)	0.04	1.41 (0.97–2.05)	0.07	1.02 (0.75–1.37)	0.91
Plaque	rs2383206	G	0.52	1.24 (0.99–1.55)	0.06	1.42 (0.96–2.09)	0.08	0.99 (0.72–1.36)	0.95
CAD, Plaque, Stroke	rs2383207	G	0.53	1.24 (0.99–1.54)	0.06	1.41 (0.96–2.06)	0.08	1.00 (0.73–1.36)	0.99
IA, AAA, CAD, PAD, Plaque	rs10757278	G	0.49	1.23 (0.99–1.53)	0.06	1.52 (1.03–2.22)	0.03	0.98 (0.72–1.34)	0.91
CAD, Plaque, Stroke	rs1333049	C	0.49	1.22 (0.99–1.50)	0.06	1.48 (1.03–2.13)	0.03	0.98 (0.73–1.31)	0.88

CAD: Coronary Artery Disease; IA: Intracranial Aneurysms; AAA: Abdominal Aortic Aneurysms; PAD: Peripheral Artery Disease; BAVM: Brain Arteriovenous Malformations.

Multivariate logistic regression model was used adjusting for age, sex, and top three principal components for ancestry.

*205 out of 338 BAVM cases had aneurysm data available