

Combination of Linkage and Association Studies for Brain Arteriovenous Malformation

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Background and Purpose—Genetic factors for brain arteriovenous malformation are unexplored because of the low incidence of familial cases, albeit local and familial clustering. We used a combination of a linkage study and an association study to explore the genetic background.

Methods—A genome-wide linkage analysis was performed in 12 patients from 6 unrelated families using the GENEHUNTER program. A genome-wide association analysis of 26 cases and 30 controls was performed using a GeneChip 10K mapping array. Significance levels for linkage and single single-nucleotide polymorphism association analyses were set at $P < 0.05$ and $P < 0.0001$, respectively. Genotyping was also performed using 58 960 single-nucleotide polymorphisms for 2 sets of discordant twins.

Results—The linkage analysis revealed 7 candidate regions, with the highest logarithm of odds score of 1.88 ($P = 0.002$) at chromosome 6q25. A significant association was observed for 4 single-nucleotide polymorphisms and 2 haplotypes, but none of them overlapped with candidate linkage regions. Genotyping of the twins showed no genetic heterogeneity.

Conclusions—The present study failed to identify genetic factors for arteriovenous malformation although the low statistical power may have resulted in such evidence being missed. (*Stroke*. 2007;38:1368-1370.)

Key Words: arteriovenous malformation ■ association ■ genome-wide ■ genetics ■ linkage ■ microarr

Brain arteriovenous malformation (AVM) is an abnormal tangle of vessels that results in arteriovenous shunting, which is an important cause of intracranial hemorrhage. Paucity of both familial and sporadic AVM¹ has hampered genetic analysis. To circumvent such difficulties, we conducted a combination of a pedigree-based linkage analysis and a population-based association analysis in the Takayama community, where both local and familial clustering has been reported.²

Materials and Methods

Subjects

The study was approved by the Ethics Committee of Kyoto University Institutional Review Board. A total of 12 patients with AVM, from 6 families (Figure: Family A,³ B,⁴ C,⁵ D,⁶ E, and F²), participated in the linkage analysis. We recruited 26 unrelated cases (17 men and 9 women) and 30 controls (14 men and 16 women) for the association analysis. Diagnosis of patients with AVM was carried out by digital subtraction angiography. All control subjects were examined by MRI and MR angiography, and none had a family history of AVM. All participants were from the Takayama community, where their families were confirmed by interview to have lived for more than 3 generations. We selected this area because of a high incidence of AVM, with an

estimated incidence of 2.4 to 3.1 in 100 000 person-years and multiple familial cases.^{2,6} Two sets of identical twins from Takayama, with discordant phenotypes, also participated.

Linkage Analysis

Genotyping for linkage analysis was performed as described previously.⁷ Because of the uncertainty of the mode of inheritance of familial AVM, both dominant and recessive models were assumed. Multipoint logarithm of odds scores were obtained using the GENEHUNTER program, version 2.0 (<http://linkage.rockefeller.edu>).⁸

Association Analysis

Genotyping was carried out using a single nucleotide polymorphism (SNP)-based GeneChip Mapping 10K 2.0 Array (10 240 SNPs; Affymetrix Inc).⁹ Association analyses were performed using GeneSpring GT2 software (Agilent Technology). The haplotype frequency was estimated by the expectation-maximization algorithm, and differences in SNP and haplotype frequencies were compared statistically using the χ^2 test.

Search for Microdeletions

Genotyping of the whole genome was conducted using a GeneChip Mapping 50K Array Xba 240 (58 960 SNPs; Affymetrix Inc) to search for genetic differences between affected and unaffected twins.

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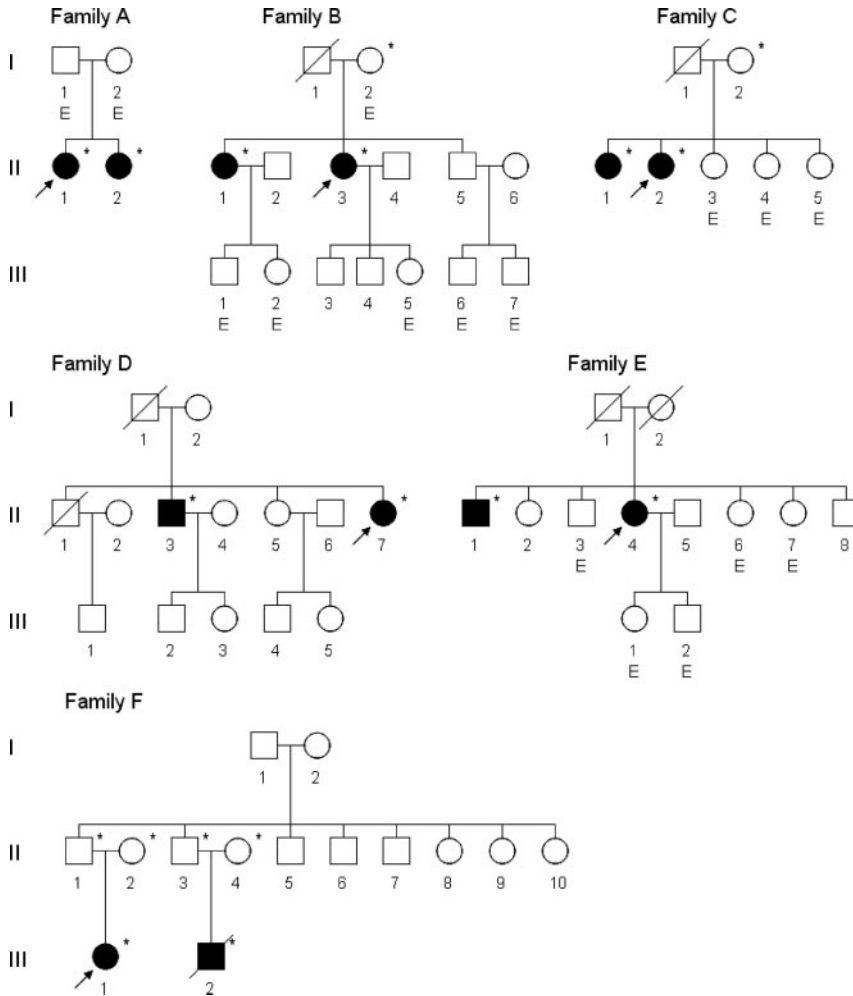
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Pedigrees of familial brain AVM. Roman numerals refer to generations, and the individuals within a generation are numbered from left to right. Filled symbols indicate patients with AVM; circles, women; squares, men; crossed symbols, deceased individuals; arrows, probands; E, participants confirmed not to have an AVM by MRI and MR angiography. * indicates a blood donor.

Statistical Criteria for the Selection of Candidate Genes

In the linkage analysis, we considered $P < 0.05$ to be an evidence of suggestive linkage. In the single SNP association analysis, $P < 0.0001$ was considered to be statistically significant. When a SNP of $P < 0.0001$ was found in the linkage regions, the overall P value of the SNP would be $< 2 \times 10^{-6}$ (0.05×0.0001), corresponding to the genome-wide significance level of 0.05 after the Bonferroni correction. For the haplotype analysis, $P < 0.0001$ after division by the number of SNPs in a haplotype was set as the level of statistical significance.

Mutation Analysis of Candidate Genes

All exons with an intron/exon boundary of ≈ 50 bp, from 4 positional candidate genes—namely, ephrin receptor B3 (*EPHB3*), ephrin B2 (*EFNB2*), protein *O*-fucosyltransferase 1 (*POFUT1*) and RUNT-related transcription factor (*RUNX2*)—were directly sequenced for the proband of each family.

Results

Linkage and Association Analysis

With a dominant model, the linkage analysis revealed 7 candidate regions (Table 1). With a recessive model, a significant P value was only obtained at 6q25 ($P = 0.005$, data not shown). The highest logarithm of odds score of 1.88 was obtained at D6S1581 with a dominant model (Table 1). Association analyses revealed 4 SNPs and 2 haplotypes with

a significant association (Table 2), none of which was in the suggestive linkage regions (Table 1). Direct sequencing *EPHB3* in 3qter, *EFNB2* in 13q32-33 and *POFUT1* in 20q11 revealed no causative variants.

Search for Microdeletions

No microdeletion was detected. Only 1 SNP (rs9296459 in 6p12) in intron 2 of *RUNX2* showed different genotypes in both sets of identical twins. However, direct sequencing of *RUNX2* failed to show any causative variants or microdeletions.

TABLE 1. List of Candidate Loci

Region	Flanking Marker	Maximum LOD Score	<i>P</i> Value
3q27	D3S1262–D3S1294	1.25	0.049
4q34	D4S621–D4S3030	1.40	0.025
6q25	D6S448–D6S1719	1.88	0.002
7p21	D7S517–D7S513	1.07	0.048
13q32-33	D13S159–D13S158	1.40	0.028
16p13-12	D16S3103–D16S3145	1.65	0.011
20q11-13	D20S195–D20S838	1.81	0.004

LOD indicates logarithm of odds.

TABLE 2. Results of Association Analyses

Analysis	Region	dbSNP ID	P value
SNP	3p12	rs1384309	0.000085
SNP	11q22	rs1938887	0.000014
SNP	18q22	rs728714	0.000053
SNP	Xp21	rs953009	0.000039
Haplotype	Xp21	rs951609	0.000011*
		rs953009	
		rs958569	
		rs1571475	
		rs1344031	
Haplotype	Xp21	rs1074193	0.000077†
		rs728149	
		rs966446	

*†The P value after division by the number of SNPs in the haplotype.

*is equal to 0.000056 divided by 5; †is equal to 0.000231 divided by 3.

Discussion

Familial occurrence of AVM is very rare. We circumvented this difficulty by increasing statistical power through the combination of linkage and association analyses. We minimized the population stratification by selecting a study population within the Takayama community, where clusters of sporadic AVM and familial AVM have been reported.⁶ However, no candidate region was found.

Using high-density SNP panels, a possible microdeletion was identified around a SNP in *RUNX2*. However, no pathological mutation in *RUNX2* was found. Although epigenetic factors were not explored, it will be worthwhile pursuing these in future studies.

The present results suggest at least 3 possibilities. First, the density of the SNP markers used might not be sufficient to detect a possible association. Although we could have reduced the required density of SNP markers by combining the association analysis with a linkage analysis, a higher density of SNP panels than was used might have been needed. Second, the number of cases used for the association analysis and the numbers of families in the linkage analysis may have been too small to obtain enough statistical power. Finally, there is a possibility that the familial AVM cases represent phenocopies.

The present study failed to identify any evidence for genetic loci for AVM. If we could double the number of sib-pairs with AVM worldwide, simulation suggests that the statistical power will increase and would reach logarithm of odds=3.6 under a dominant model. A consortium will be established to recruit familial AVMs.

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Disclosures

None.

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