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 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. 

Key Words: arteriovenous malformation association genome-wide genetics linkage microarray

Blood 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 AVM 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.

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, B, C, D, E, and F), 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 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.

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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 $<2 \times 10^{-6}(0.05 \times 0.0001)$, 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 $\sim 50$ 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**

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

LOD indicates logarithm of odds.
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. However, no candidate region was found. Using high-density SNP panels, a possible microdeletion was identified around a SNP in \textit{RUNX2}. However, no pathological mutation in \textit{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.

**References**


**TABLE 2. Results of Association Analyses**

<table>
<thead>
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<th>dbSNP ID</th>
<th>$P$ value</th>
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<td>SNP</td>
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<td>0.000011*</td>
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<td>rs966446</td>
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</table>

*†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.
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