Genomewide Linkage in a Large Caucasian Family Maps a New Locus for Intracranial Aneurysms to Chromosome 13q

Teresa Santiago-Sim, PhD; Steven R. DePalma, PhD; Kevin L. Ju; Barbara McDonough, RN; Christine E. Seidman, MD; J.G. Seidman, PhD; Dong H. Kim, MD

Background and Purpose—Familial aggregation of intracranial aneurysms (IAs) indicates a genetic role in the pathogenesis of this disease. Despite a number of reported susceptibility loci, no disease-causing gene variants have been identified. In this study, we used a parametric genomewide linkage approach to search for new IA susceptibility loci in a large Caucasian family.

Methods—The affection status of family members with clinical signs of IA was confirmed with medical records or through radiological or surgical examinations. All other relatives were screened using MR angiography. Genomewide linkage analysis was performed on 35 subjects using approximately 250 000 single nucleotide polymorphic markers.

Results—Ten individuals had an IA. Linkage analysis using a dominant model showed significant linkage to a 7-cM region in 13q14.12–21.1 with a maximum logarithm of odds score of 4.56.

Conclusion—A new IA susceptibility locus on 13q was identified, adding to the number of IA loci already reported. Given that no coding variants have been reported to date, it is possible that alternative genetic variants such as regulatory elements or copy number variation are important in IA pathogenesis. We are proceeding with attempts to identify such variants in our locus. (Stroke. 2009;40[suppl 1]:S57-S60.)

Key Words: genetics ■ intracranial aneurysm ■ linkage

A genetic basis of intracranial aneurysm (IA) formation has long been noted. Up to 20% of patients with IA have a positive family history1–3 and there is up to a 7-fold increased risk of IA rupture among first-degree relatives compared with second-degree relatives.4 Although many genetic loci have been linked to familial IA,5–15 no single disease-causing gene variant has been identified. In this study, we performed genomewide linkage analysis on a single family to identify an IA susceptibility locus.

Materials and Methods

Subjects

The subject of this study is a Caucasian family (CVM family) of French-Canadian heritage (Figure 1). Thirty-two first-degree relatives and 3 unrelated spouses were enrolled after providing written consent. The clinical status of affected individuals was confirmed through radiological or surgical investigations and with medical records. All living first-degree relatives at least 18 years of age were invited for noninvasive MR angiography screening at the Longwood MRI facility (Massachusetts). For subjects with positive MR angiography results, further screening was sought using conventional cerebral angiography to determine the need for treatment. Individuals with negative MR angiography results and spouses of family members were scored as unaffected. Unscreened individuals were scored as unknown. Blood or saliva samples were collected and genomic DNA was extracted using standard methods or using DNA purification kits (OraGene). This study was approved by the Institutional Review Boards at the University of Texas Health Science Center at Houston and the Brigham and Women’s Hospital in Boston.

Linkage Analysis

Genomewide linkage analysis was performed on 35 subjects (Figure 1) with the Affymetrix GeneChip Human Mapping 250K Sty1 Array (Affymetrix, Inc, Santa Clara, Calif). This platform contains 238 304 single nucleotide polymorphism markers distributed throughout the genome with an average spacing of approximately 12 kb. Samples were prepared using the Affymetrix 250K Sty Assay Kit according to the manufacturer’s protocol. Logarithm of odds (LOD) scores were calculated with Vitesse and LinkMap softwares. LOD scores were calculated using a 4-marker sliding window on a Linux computing cluster, and single nucleotide polymorphism allele frequencies were fixed at 0.5/0.5. Autosomal-dominant inheritance and a disease gene frequency of 0.001 were assumed. Phenocopy rate was set to zero. Subjects were classified into 2 liability classes with penetrance set at 95% for subjects at least 50 years of age and at 15% for those younger than age 50.

Results

Clinical Evaluations

The CVM family (Figure 1) was identified when the proband (III-9) presented with a subarachnoid hemorrhage from a
ruptured IA and was successfully treated by the senior author (D.H.K.). Several family members have displayed clinical signs and symptoms of IA and have also undergone aneurysm repair. Two of her own children (IV-15 and IV-17) have had aneurysmal subarachnoid hemorrhage while in their second or third decade of life. One of her nieces (IV-6) was diagnosed with IA, cerebrovascular infarct, and a carotid dissection at the age of 51. An incidental IA was found in V-26 in a head scan at the age of 60 after reporting poor balance that was thought to be secondary to her cancer radiation treatment. MR angiography was performed for 22 other relatives, identifying 5 additional affected members (IV-9, IV-10, IV-12, IV-14, and IV-22), mostly in their fourth or fifth decade of life (Table). IA inheritance is consistent with an autosomal-dominant pattern. No evidence of aneurysm was found for IV-12, suggesting decreased penetrance for this disorder. The actual cause of death of III-3 or III-6 is unknown.

Mapping of the Intracranial Aneurysm Locus
Genomewide linkage analysis results are shown in Figure 2. Significant linkage was obtained for a 7-cM region (flanked by markers rs7983420 and rs17054625) on 13q14.12–21.1 with a maximum multipoint LOD score of 4.56 (Figure 2B–C). LOD scores suggestive of linkage were also obtained for a 35-cM region in 5q22.2–33.3 (LOD 2.91, Figure 2D), a 3.5-cM region in 9p23 (LOD 2.93, Figure 2E), a 4-cM region on 12p12 (LOD 3.10, Figure 2F), and a 10-cM region on 18p11 (LOD 3.15, Figure 2G). Interestingly, the region in 5q overlaps with a possible IA susceptibility region (5q22–31) identified in a nonparametric linkage analysis in Japan. However, high LOD scores in 5q, 9p, 12p, and 18p were intermittent and interrupted by highly negative scores (Figure 2D–G). Sixty-six percent of single nucleotide polymorphisms were excluded (LOD <2.0), including markers at previously reported IA susceptibility loci in 1p34.3–36.13, 2p13, 5p15.2, 7q11, 11q24–25, 14q23–31, 17cen, 19q13.3, and Xp22.2–22.3.

Discussion
The identification of hundreds of families with IA suggests that genetic factors are involved in IA formation. Several approaches such as a candidate gene approach and genomewide parametric or nonparametric studies have been used to look for IA susceptibility genes. Genes encoding extracellular matrix components such as elastin, collagen, and fibrillin have been popular candidate genes and have been widely studied. However, association tests performed on variants in these genes produced negative or inconsistent results. The use of a nonparametric linkage approach has identified several putative IA susceptibility loci, suggesting the presence of genetic heterogeneity in this disorder. To minimize the effects of genetic heterogeneity that may lead to ambiguous results, we used an alternative parametric linkage approach on a single, large Caucasian family. Linkage analysis on the CVM family using a dominant model produced several loci suggestive of linkage. However, the most striking linkage region obtained was a 7-cM locus on 13q14.12–21.1 with a highly significant LOD score of 4.56. Haplotype analyses with microsatellite markers will help confirm or exclude these regions.

We have identified a new and highly significant susceptibility locus in 13q, which contains approximately 60 genes. However, no coding variants have been reported to date for any IA susceptibility locus. Therefore, it is possible that alternative genetic variants such as regulatory elements or copy number variation are important in IA pathogenesis. We

Table. Clinical Features of Affected CVM Members

<table>
<thead>
<tr>
<th>Subject</th>
<th>Current Age, Years</th>
<th>Age at Diagnosis, Years</th>
<th>Sex</th>
<th>Aneurysm Location</th>
<th>Repaired</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-9</td>
<td>75</td>
<td>73</td>
<td>F</td>
<td>OpA</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>IV-6</td>
<td>55</td>
<td>51</td>
<td>F</td>
<td>LMCA</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>IV-9</td>
<td>47</td>
<td>45</td>
<td>F</td>
<td>RMCA</td>
<td>N</td>
<td>F</td>
</tr>
<tr>
<td>IV-10</td>
<td>45</td>
<td>44</td>
<td>M</td>
<td>RACoA</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>IV-12</td>
<td>48</td>
<td>47</td>
<td>F</td>
<td>RPCoA</td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>IV-14</td>
<td>54</td>
<td>52</td>
<td>M</td>
<td>Nine aneurysms</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>IV-15</td>
<td>53</td>
<td>35</td>
<td>M</td>
<td></td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>IV-17</td>
<td>48</td>
<td>27</td>
<td>M</td>
<td>ACoA</td>
<td>Y</td>
<td>S</td>
</tr>
<tr>
<td>IV-22</td>
<td>46</td>
<td>45</td>
<td>M</td>
<td>RATemp</td>
<td>N</td>
<td>F</td>
</tr>
<tr>
<td>IV-26</td>
<td>68</td>
<td>60</td>
<td>F</td>
<td>RMCA</td>
<td>Y</td>
<td>S</td>
</tr>
</tbody>
</table>

OpA indicates ophthalmic artery; LMCA, left middle cerebral artery; RMCA, right middle cerebral artery; RACoA, right anterior communicating artery; RPCoA, right posterior communicating artery; RATemp, right anterior temporal artery; Y, yes; N, no; S, saccular; F, fusiform.
are proceeding with attempts to identify such variants in our locus in addition to further positional cloning for identification of coding variants.

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**Disclosures**

None.

**References**

haemorrhage in first and second degree relatives of patients with sub-arachnoid haemorrhage. BMJ. 1995;311:288–289.
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