Genome-Wide Linkage in a Large Dutch Consanguineous Family Maps a Locus for Intracranial Aneurysms to Chromosome 2p13

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Background and Purpose—Familial occurrence of intracranial aneurysms suggests a genetic factor in the development of these aneurysms. In this study, we present the identification of a susceptibility locus for the development of intracranial aneurysms detected by a genome-wide linkage approach in a large consanguineous pedigree.

Methods—Patients with clinical signs and symptoms of intracranial aneurysms, confirmed by radiological, surgical, or postmortem investigations, were included in the study. Magnetic resonance angiography was used to detect asymptomatic aneurysms in relatives.

Results—Seven out of 20 siblings had an intracranial aneurysm. Genome-wide multipoint linkage analysis showed a significant logarithm of the odds score of 3.55.

Conclusion—In a large consanguineous pedigree intracranial aneurysms are linked to chromosome 2p13 in a region between markers D2S2206 and D2S2977. (Stroke. 2004;35:2276-2281.)

Key Words: genetics ■ intracranial aneurysm ■ subarachnoid hemorrhage

Rupture of an intracranial aneurysm (IA) causing a subarachnoid hemorrhage (SAH) occurs with a frequency of between 6 and 8 per 100 000 in most Western populations. Up to 25% of IA cases occur in relatively young patients compared with other types of stroke, on average between 50 and 64 years of age. Despite improvements in medical and surgical management during the past decades, aneurysmal SAH is still a major public health problem with a morbidity and mortality rate of ~50%. Inherited susceptibility probably plays an important role in the development of IA. Several studies described familial occurrence of intracranial aneurysms and 1 study described a very high concordance (87.5%) of IAs in monozygotic twins. The risk of rupture appears to be associated with, besides genetic factors, several environmental risk factors like smoking and alcohol consumption. The risk of rupture in first-degree relatives of patients with aneurysmal SAH is 4-times higher, and in siblings 6-times higher, than in the general population. When screened by magnetic resonance angiography, 4% of the first-degree relatives of sporadic patients with aneurysmal SAH were shown to have intracranial aneurysms.

Both autosomal recessive and autosomal dominant patterns of inheritance have been described, which indicate genetic heterogeneity. Genetic studies of IA that have been published so far show no overlapping regions with suggestive linkage, underlining the genetic heterogeneity. In the present study, we performed a genome-wide linkage analysis in a large consanguineous pedigree to identify a susceptibility locus for IA.

Patients and Methods

Clinical Diagnosis and Definitions

The subject of investigation was a single consanguineous family with IA in 1 generation (Figure 1). In addition, 4 other nonconsanguineous families, selected on the basis of simulation studies, were studied in a separate analysis.

In patients with clinical signs and symptoms of IA, confirmation was established through radiological, surgical, or postmortem investigations. Aneurysms were designated as "confirmed" symptomatic when either an aneurysmal SAH had occurred (confirmed at com-
puted tomography (CT) scan and cerebral angiography or at autopsy) or neurological impairment was caused by an angiographically visualized aneurysm, i.e., a mass effect of the aneurysm. Patients with a clinically suspected aneurysmal SAH but without verification on CT scan and subsequent cerebral angiography or at autopsy were designated as "suspected" symptomatic. Patients with a clinically suspected aneurysmal SAH but without verification on CT scan and subsequent cerebral angiography or at autopsy were designated as "suspected" symptomatic. Aneurysms that were found during screening were designated as asymptomatic.

Screening
All living first-degree relatives aged 18 years or older were invited to be screened by means of noninvasive magnetic resonance angiography (MRA). In patients in whom MRA was positive for IA, confirmation was sought using conventional cerebral angiography to ascertain whether surgery or coiling of the aneurysm was required. Screening was repeated after 5 years in relatives whose initial screens were negative. In patients with aneurysms, the treating physician and a clinical geneticist ascertained that these aneurysms were not associated with other intracranial vascular or known heritable diseases (e.g., arteriovenous malformation, autosomal dominant polycystic kidney disease, or Ehlers–Danlos syndrome).

Linkage Analysis
After informed consent was obtained, blood samples were drawn for DNA extraction. DNA was extracted with DNAzol reagent according to the manufacturer’s instructions (Invitrogen). For genome-wide linkage analysis, a polymorphic marker set was used (screening set 6; Marshfield clinic, Marshfield Wis; see http://www.marshfield.org/research/genetics/sets). These markers, with an average distance of 10 centimorgans, were labeled (Isogen Life Science) for detection on an ABI 310 sequencer (Applied Biosystems). In areas of suggestive linkage, additional markers were used that were derived from the Genethon database (www.genethon.fr). Allele frequencies of markers for the Western European population were obtained from the Genethon database. Genome-wide analysis was performed with the initially known 5 surviving patients (IV-7, IV-11, IV-14, IV-18, and IV-22) and the unaffected mother (III-1) of the family described in Figure 1. All markers for which the 5 patients were homozygous for identical alleles were also analyzed in 5 unaffected siblings. If the unaffected siblings were homozygous for the same allele, the marker was considered to be uninformative.

In a subsequent analysis, all remaining informative regions were investigated further by fine mapping with additional markers. Because an IA was detected during follow-up screening in individual IV-15, this individual was included as "affected" in the subsequent fine mapping studies. The partner and 5 children of the deceased patient IV-2 were included to allow reconstruction of her genotype. Patient IV-2 died of a suspected SAH before the study and her DNA was not available. In the logarithm of the odds (LOD) score calculations, the clinical phenotype of her children was "unknown," because the children of this patient were too young to be affected.

Additional Analysis of Other Families
After linkage was established in the described consanguineous pedigree, we selected 4 other unrelated families from our series of 51 pedigrees with familial IA. The selection of these families was based on simulation studies that showed that they could be informative for linkage with an expected LOD score of >1 for each family. Markers D2S370, D2S2206, D2S136, D2S2293, D2S380, D2S291, and D2S286 were used for this additional analysis.
LOD score analyses were performed using SIMWALK 2.86.24,25 Because of the consanguinity in the pedigree, parametric testing was performed using a recessive mode of inheritance and a reduced penetrance of 70%. The frequency of IA in the general population younger than age 50 was set at 1%. Based on angiography and autopsy studies,22,26 2% to 3% of the population have an asymptomatic IA. Because there are insufficient data available to establish an exact age-dependent penetrance, calculations were also performed assuming 80% and 90% penetrance. For the genome-wide screening, the significance criteria were used according to the guidelines of Lander and Kruglyak.27

Ethical Considerations
Our local ethics committee approved the study protocol. All relatives of the described pedigrees received comprehensive information about the genetic aspects of the study and consented to blood sampling for DNA analysis.

Results
Details of the pedigree studied are given in Figure 1 and the Table. The parents (generation III) are first cousins. In the fourth generation, 7 of 20 siblings were affected at the conclusion of our study. The father (II-1) died at age 62 of a cause unrelated to IA. One sibling died of unknown causes soon after birth (IV-10). Two siblings (IV-1 and IV-9), not symptomatic until at least the age of 84 and 74, respectively, declined participation (both MRA screening and DNA sampling) in the study. Of the remaining 17 siblings, the Table shows that in 3 an aneurysmal SAH had occurred at 37 (IV-11), 42 (IV-7) and 45 (IV-14) years of age. One sibling (IV-2) had clinical signs and symptoms of aneurysmal SAH at the age of 36 and died before a definite diagnosis could be established. No autopsy was performed; therefore, this patient was designated as “suspected” SAH. Because no DNA was available from this patient, her genotype was reconstructed by means of the genotype of 5 of her 6 children, her partner (the father of the children), and her mother. MRA screening of the mother (age 83) and the 6 children of patient IV-2 (ages 32, 33, 36, 37, 38, and 40) revealed no IA. Screening of the 13 asymptomatic siblings revealed cerebral aneurysms in 3 siblings at ages 38 (IV-22), 45 (IV-18), and 52 (IV-15). Sibling IV-15 was initially unaffected, but during follow-up MRA screening an asymptomatic aneurysm was found.

The initial genome-wide screening of the mother (III-2) and 5 affected siblings who were known as affected at the start of the study showed 2 regions, 1 on chromosome 2 and another on 5, with suggestive linkage with multipoint LOD scores of 2.94 for the region around D2S1394 and 3.23 around marker D5S496 (Figure 2).

These 2 regions were characterized in more detail using additional markers for fine mapping. The unaffected siblings and the partner and 5 children of the deceased patient were included in this analysis. Sibling IV-15 was included as affected in this analysis. Taking into account the consanguinity in the pedigree and assuming recessive inheritance with a

<table>
<thead>
<tr>
<th>Patient ID and Birth Year</th>
<th>Sex</th>
<th>Age at Last Screening or First Symptoms, y</th>
<th>Symptomatic, Asymptomatic or Negative Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV-1, 1928 M</td>
<td>Not symptomatic, did not further participate in study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-2, 1931 F</td>
<td>36</td>
<td>S*</td>
<td></td>
</tr>
<tr>
<td>IV-4, 1932 M</td>
<td>61</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-5, 1933 F</td>
<td>65</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-6, 1935 F</td>
<td>59</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-7, 1936 M</td>
<td>42</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>IV-9, 1938 M</td>
<td>Not symptomatic, did not further participate in study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-10, 1939 F</td>
<td>59</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-11, 1940 M</td>
<td>37</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>IV-12, 1942 F</td>
<td>Died soon after birth of unknown cause</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-13, 1943 F</td>
<td>55</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-14, 1945 F</td>
<td>45</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>IV-15, 1946 F</td>
<td>47</td>
<td>AS</td>
<td></td>
</tr>
<tr>
<td>IV-16, 1947 M</td>
<td>51</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-18, 1949 F</td>
<td>45</td>
<td>AS</td>
<td></td>
</tr>
<tr>
<td>IV-19, 1950 M</td>
<td>48</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-20, 1952 M</td>
<td>47</td>
<td>—</td>
<td></td>
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<tr>
<td>IV-21, 1953 M</td>
<td>40</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>IV-22, 1955 F</td>
<td>38</td>
<td>AS</td>
<td></td>
</tr>
<tr>
<td>IV-23, 1959 F</td>
<td>40</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

S indicates patients with symptomatic IA (SAH in all of them); S*, patient with a suspected symptomatic IA (SAH), ie, clinical signs and symptoms of aneurysmal SAH but without proven aneurysm at angiography or autopsy; AS, patients in whom screening revealed an asymptomatic IA; —, patients in whom screening revealed no IA.
penetrance of 70%, the multipoint LOD score decreased for chromosome 5 to 1.78 and increased to 3.55 for chromosome 2, with the maximum near marker D2S2293. Varying the penetrance to 80% and 90% resulted in similar LOD score curves. The LOD score decreased sharply distal of D2S2206 and proximal of D2S2977 because of recombinations in these regions (Figure 3).

After the locus was found in the described consanguineous pedigree, linkage was tested on 2p13 in 4 other unrelated families with familial IA in an additional analysis. Assuming a single gene responsible for IA in these 4 families (the homogeneity parameter alpha, indicating the proportion of linked families, was set to 1 in SIMWALK), the maximum multipoint LOD score was −1.212. The multipoint LOD scores were negative in 3 families and positive (+0.9) in 1. Calculations for all families together, including the large consanguineous pedigree, with a variable homogeneity parameter alpha resulted in a maximum multipoint LOD score of 2.98 near marker D2S2293 and an alpha value of 0.35.

**Discussion**

The pedigree investigated in this study concerned a known consanguineous family (parents were first-cousins). It is obvious that within a single consanguineous pedigree like the current one, genetic heterogeneity is highly unlikely. Consequently, for the purpose of LOD score calculations, the intracranial aneurysms in this family were considered a monogenic disorder with a recessive mode of inheritance and reduced penetrance.

In this family, linkage was found on chromosome 2p13. The multipoint LOD score reached a maximum of 3.55 (θ=0) near marker D2S2293, reaching genome-wide significance for a monogenic disorder. To investigate whether the linkage found in this pedigree suggests a common locus in familial IA, additional linkage analysis was performed in 4 other unrelated pedigrees with familial IA. No evidence of linkage at 2p13 was found in 3 of these families, suggesting genetic heterogeneity. One family yielded a positive multipoint LOD score of 0.9 in the 2p13 region, indicating a possible linkage with this locus. When combining the results of all families, including the large consanguineous pedigree, the calculations showed that a maximum LOD score was reached for a proportion of 0.35 linked families.

Until now, the genetics of IA were largely unknown. Detailed analyses in large numbers of pedigrees failed to identify a single mode of inheritance. Several authors have described IA associated with other diseases but no specific gene. IA are common in polycystic kidney disease, which is linked to chromosomes 16 and 4. Intracranial aneurysms have never before been linked to a locus at chromosome 2p13. Earlier studies showed linkage on other chromosomes. In a genome-wide linkage analysis in 104 Japanese sib-pairs of patients with confirmed IA, Onda et al found linkage on chromosome 7q11 with a maximum multipoint LOD score of 3.22. In another study in 48 Finnish sib-pairs with IA, linkage was found on chromosome 19q13. Additional regions with suggestive linkage were found in both studies, but there was no overlap between the regions identified by the
studies and neither study found any evidence for linkage on 2p13.18 Our findings that only one-third of the families show linkage to 2p13 also indicate locus heterogeneity. We have demonstrated that in 1 large Dutch pedigree, IA is linked to chromosome 2p13 in a region between D2S2206 and D2S2977. This region encompasses 7 centiMorgans and contains ~150 genes. It does not contain any of the previously suspected and studied candidate genes.28,32 For instance, the collagen type III gene (COL3A1), situated on 2q31, was excluded by our linkage analysis.33

In general, candidate genes, probably involved in the development of IA, can be roughly divided into genes that encode proteins of the extracellular matrix or genes involved in development and growth.28,34–37 Screening the region found in our pedigree, several of these candidate genes can be identified.

For instance, several studies showed involvement of smooth muscle actin abnormalities in cerebral aneurysm walls, consequently, genes encoding proteins of the actin metabolism, like smooth muscle actin gamma 2 (ACTG2) and actin-related protein 2 (ARP2 / ACTR2), are potential candidates.38 Another interesting candidate gene is BMP10, encoding the bone morphogenic protein 10. A related protein, bone morphogenic protein receptor 2 (BMPR2), is involved in the vascularization of the retina and the epidermal growth factor-containing family.39 Other candidate genes are the ventral anterior homeobox 2 gene (VAX2), playing a role in the vascularization of the retina and the epidermal growth factor containing fibrin-like extracellular matrix protein 1 (EFEMP1) gene, involved in the retinal disease Malattia Leventinese. Transforming growth factor alpha gene (TGFA) and tumor endothelial marker 8 gene (TEM8) are also interesting candidate genes that merit further investigation.36

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References


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