Stroke Genetics Update
Mark J. Alberts, MD

The genetics of cerebrovascular disease is an area of increasing interest and advances. During the past year there have been a number of significant discoveries in the area that have increased our knowledge and understanding of the importance of genetics factors in the epidemiology of, etiology of, pathophysiology of, and recovery from cerebrovascular disease. This review will highlight some of the publications that have advanced our knowledge in these areas.

Genetic Linkage Studies
One of the most significant advances in understanding stroke etiology and genetics was reported by the deCode group based in Iceland. In that country, there exist medical data on many multigenerational families with a variety of common disorders (ie, heart disease, stroke, diabetes, asthma). Using these extensive family resources, the deCode researchers conducted a genome-wide screen on 179 pedigrees with at least 2 members having mostly ischemic strokes. Patients with subarachnoid hemorrhage were excluded, but a few patients with intracerebral hemorrhage were included in the initial screen. Evidence for linkage was found to a region of chromosome 5 (5q12) with a logarithm of odds (LOD) score of 4.40 at marker D5S2080. When the patients with intracerebral hemorrhage were excluded, the LOD score increased to 4.86 at the same locus.1

There are many possible candidate genes in the area of established linkage. The researchers have indicated that they have identified a very promising gene within this region that has an important function related to vascular stability. It will be quite interesting to see the actual identity and function of this gene.

Despite these positive and exciting results, some degree of caution is urged before we can conclude that this putative gene is the root cause of most cases of ischemic stroke. First, the Icelandic population is relatively isolated and has a distinct gene pool. The results of the deCode study may not be applicable to other populations. Indeed, studies are underway to duplicate the deCode results in populations different from those in Iceland.

Another issue is that the deCode study lumped together all types of ischemic stroke, including thrombotic, embolic, large vessel, and small vessel. Although the end result of all these stroke mechanisms is the same, they tend to affect blood vessels in somewhat different ways. It will be quite interesting to see whether the “stroke gene” identified by the deCode group is involved in all of these diverse mechanisms.

Genetic Epidemiology Studies
A recent epidemiology study of stroke made use of the Dutch Twin Registry. This long-term study found that 10% of monozygotic twins were concordant for stroke death, compared with 5% of dizygotic twins. Further analyses showed heritability indices of 0.32 for stroke death and 0.17 for stroke hospitalization.2 These data suggest a moderate genetic effect for stroke, which is consistent with prior studies.

Another recent study examined the genetic epidemiology of ICH. This study found that 10% of probands had a positive family history of ICH. Four pedigrees had more than 2 patients with an ICH. There were no significant demographic or clinical differences between the familial and nonfamilial cases.3

Another hospital-based study of ICH found that that apolipoprotein E alleles e2 and e4 were associated with the occurrence of lobar hemorrhages. A first-degree relative with ICH was also a risk factor for lobar ICH.4

Performing family studies of stroke represents significant challenges in study design, patient ascertainment, and genetic analyses. A study by Hassan and colleagues5 used family history data and statistical modeling to estimate the sample sizes needed for various genetic studies of stroke (Table 1). This study found that in general one would have to screen several thousand stroke patients to ascertain several hundred affected sib-pairs, which would be needed to have a reasonable chance of detecting a significant genetic association.

The ongoing SWISS study, under the direction of Dr. James Meschia, has been successful in identifying several hundred sib-pairs with ischemic stroke. This will be a valuable resource for identifying genes that are important in stroke pathogenesis.

Genetic Polymorphisms and Candidate Genes
The identification of CADASIL (cerebral autosomal dominant arteriopathy, subcortical infarcts, and leukoencephalopathy) as a fairly common genetic etiology for ischemic stroke has been a significant advance. CADASIL is a result of a variety of mutations in the NOTCH3 gene, which is responsible for cell signaling and vascular development.6 A number of polymorphisms in the NOTCH3 gene have been identified. Recently a Japanese group studied one of the NOTCH3 polymorphisms (T6746C) in 235 patients with sporadic ischemic stroke and 315 controls. They did not find a significant association between the polymorphism and ischemic stroke.7
Animal models of inherited stroke, particularly the spontaneously hypertensive stroke-prone rat, have been used to identify candidate genes. One of the genes identified using this model is the atrial natriuretic peptide. In a large case-control study involving 970 Japanese subjects, there was no evidence that the atrial natriuretic peptide gene or surrounding markers were associated with stroke.\(^{8}\)

A number of recent studies have examined various collagen genes in patients with dissection of the cervical arteries. One study examined the collagen 3A1 gene in patients and one family with cervical dissections. Using both direct sequencing and linkage studies, there was no evidence that the COL3A1 gene was involved in the pathogenesis of dissections in these patients.\(^{9}\) Another study examined a small family with carotid dissection and connective tissue abnormalities by electron microscopy. Using a linkage exclusion approach, they were able to exclude 34 different genes involved in the extracellular matrix.\(^{10}\)

Another study of 10 patients with cervical dissections examined the alpha2 chain of type V procollagen as a candidate gene. Two different missense mutations were detected in two patients, and a third patient had two more mutations in the alpha2 (V) chain. Some of these mutations were also found in 50 control subjects.\(^{11}\)

A growing list of polymorphisms continues to be associated with stroke in humans. A recent review of polymorphism association genetic studies highlights some of the problems with such studies.\(^{12}\) These problems include lack of adequate sample size, the use of specific populations that may skew the results, ascertainment biases, unmatched control populations, and the important fact that in most cases the polymorphism does not seem to affect the expression of the gene or function of the protein. A list of recently reported polymorphism association studies for stroke is provided in Table 2.

In keeping with the limitations of such polymorphism studies, a study investigated the association between genetic polymorphisms in the CD14 lipopolysaccharide receptor gene (C-260T) and the CD18 leukocyte adhesion molecule (codon 441). A total of 338 affected patients and 338 controls were studied. There was no evidence of a significant association for either gene using a dominant or recessive model.\(^{13}\)

A recent study investigated the effect of apolipoprotein E genotype on outcome after a subarachnoid hemorrhage.\(^{14}\) Of the 72 patients in the study, 15% had an apoE e4 allele. Six-month outcomes were worse in patients with the e4 allele using the Glasgow Outcome Scale. This effect persisted after using a multiple logistic regression model (\(P=0.003\)).

Elevated homocysteine levels (hyperhomocysteinemia) continue to be reported as a significant risk factor for ischemic stroke, especially in young patients. A recent meta-analysis suggests a hazard ratio of 1.79 for elevated homocysteine and ischemic stroke.\(^{15}\) The role of the TT polymorphism remains unclear. Although this genotype may predispose individuals to an elevated homocysteine level, there are clearly other factors that affect homocysteine levels and hence stroke risk.\(^{16}–^{17}\)

Cerebral cavernous malformations (CCM) are often inherited. In many familial cases, CCM is a result of mutations in the KRIT-1 gene.\(^{6}\) The precise mechanism by which mutations in KRIT-1 produce CCMs is unclear. One model suggests a two-hit mechanism, in which one mutation is inherited and the other occurs in somatic cells, or two somatic mutations occur. A study of a sporadic cavernoma case found evidence for two somatic mutations.\(^{18}\)

The September 2002 (Volume 32) issue of *Nature Genetics* is devoted to a “user’s guide to the human genome.” It is a

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### TABLE 1. Sample Size Estimates for Genetic Studies

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Case</th>
<th>Control</th>
<th>TDT</th>
<th>S-TDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases screened</td>
<td>820</td>
<td>31 680</td>
<td>3062</td>
<td></td>
</tr>
<tr>
<td>Final sample size</td>
<td>414</td>
<td>414</td>
<td>617</td>
<td></td>
</tr>
</tbody>
</table>

TDT, indicates transmission disequilibrium testing; s-TDT, sibling transmission disequilibrium testing.

Data adapted from Reference 5.

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### TABLE 2. Recent Polymorphisms Studied in Cerebrovascular Disease

<table>
<thead>
<tr>
<th>Gene/Protein</th>
<th>Stroke Type</th>
<th>Polymorphism</th>
<th>Association</th>
<th>Significance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XIII</td>
<td>ICH</td>
<td>Val34Leu</td>
<td>No</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>ACE</td>
<td>Leukoaraiosis</td>
<td>I/D</td>
<td>Yes, for DD</td>
<td>0.02</td>
<td>20</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Stroke</td>
<td>4G/5G I/D</td>
<td>No</td>
<td>—</td>
<td>21</td>
</tr>
<tr>
<td>NOTCH3</td>
<td>Stroke</td>
<td>T6748C</td>
<td>No</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>ANP</td>
<td>Stroke</td>
<td>G664A</td>
<td>No</td>
<td>—</td>
<td>22</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Lacunar</td>
<td>G(−174)C</td>
<td>Yes</td>
<td>0.03</td>
<td>23</td>
</tr>
<tr>
<td>Paraononase</td>
<td>Ischemic</td>
<td>Q192R, L55M</td>
<td>Yes, 192R</td>
<td>0.001</td>
<td>24</td>
</tr>
<tr>
<td>ApoE</td>
<td>SAH-outcome</td>
<td>e4</td>
<td>Yes</td>
<td>0.003</td>
<td>14</td>
</tr>
<tr>
<td>CD14</td>
<td>Stroke</td>
<td>C(−260)T</td>
<td>No</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>CD18</td>
<td>Stroke</td>
<td>Codon 441</td>
<td>No</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>Fibrinogen-β</td>
<td>Ischemic stroke</td>
<td>A(−445)G</td>
<td>Yes, men only</td>
<td>0.02</td>
<td>25</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>Ischemic stroke</td>
<td>S291, X447</td>
<td>Yes, X447, men only</td>
<td>0.01</td>
<td>26</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>Stroke</td>
<td>m5178C/A</td>
<td>Yes</td>
<td>&lt;0.01</td>
<td>27</td>
</tr>
</tbody>
</table>

ICH indicates intracerebral hemorrhage; I/D, insertion, deletion polymorphism; PAI-I, plasminogen activator inhibitor-I; ANP, atrial natriuretic peptide.
very practical yet powerful primer on how to access many of the data generated by the various projects involved in sequencing the human genome.

Conclusion
The field of stroke genetics continues to grow and expand in many directions. Most of the major advances to date have been made by studying specific and well-defined stroke subtypes or particular disease mechanisms. Association polymorphism studies have had only limited success and face challenges related to sample size and disease heterogeneity. We are hopeful that the coming year will produce even more success in identifying genetic factors responsible for stroke and cerebrovascular disease.

References

*KEY WORDS*: genetics ■ mutation ■ polymorphism
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