Whole Genome Analyses Suggest Ischemic Stroke and Heart Disease Share an Association With Polymorphisms on Chromosome 9p21

Mar Matarin, PhD; W. Mark Brown, MA; Andrew Singleton, PhD; John A. Hardy, PhD; James F. Meschia, MD; for the ISGS investigators

Background and Purpose—Recently independent studies reported an association between coronary heart disease and single-nucleotide polymorphisms (SNPs) located at chromosome 9p21, near CDKN2A and CDKN2B genes. Given that stroke is a common complication after myocardial infarction, we investigated if the same SNPs were associated with ischemic stroke in our population.

Methods—We recently initiated a whole genome analysis of ischemic stroke and published the first stage of a case control study using >400,000 SNPs from Illumina Infinium Human-1 and HumanHap300 assays. We focused on SNPs recently associated with heart disease by Helgadottir and colleagues and SNPs from the same haplotype block.

Results—In analyses both unadjusted and adjusted for stroke risk factors, significant associations with ischemic stroke were observed for SNPs from the same haplotype block previously associated with myocardial infarction. Significant association was also seen between disease and haplotypes involving these SNPs, both with and without adjustment for stroke risk factors (odd ratios: 1.01 to 2.65).

Conclusions—These data are important for 3 reasons: first, they suggest a genetic association for stroke; second, they suggest that this association shares pathogenic mechanisms with heart disease and diabetes; and third, they illustrate, that public release of data can facilitate rapid risk locus discovery. (Stroke. 2008;39:1586-1589.)

Key Words: ischemic stroke ■ genetics ■ heart disease ■ diabetes

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ecently, independent studies have reported an association between myocardial infarction (MI) and common genetic variation on chromosome 9p21. The variants associated with MI are located in a linkage disequilibrium (LD) block near the genes CDKN2A and CDKN2B.1-4 At the same time, genetic variants around the same genes were associated with an increased risk of type 2 diabetes mellitus5-7 and atherothrombosis.8 All these data suggest there may be common pathogenic mechanisms involved in these apparently disparate diseases.

Coronary disease increases the risk of stroke, and stroke is associated with a large increase in the risk for death after MI. Furthermore, both diseases share common risk factors and treatments9; because of this we sought to determine whether the CDKN2A/CDKN2B locus associated with MI may also modulate risk for IS. To do this we analyzed data from our recently completed first stage of a whole genome analysis of IS that used more than 400,000 single-nucleotide polymorphisms (SNPs) from Illumina Infinium Human-1 and HumanHap300 assays, on a cohort of 249 samples with ischemic stroke (IS) and 268 controls.10 Here we present the results of these analyses.

Subjects and Methods

Participants

All stroke samples used in the present study were collected by the Ischemic Stroke Genetics Study (ISGS), which is a prospective 5-center North American case-control study. Control samples were from the National Institute of Neurological Disorders and Stroke Neurogenetics Repository. The protocol for ISGS and controls has been reported previously.10,11 Briefly, stroke was defined according to World Health Organization criteria, and index strokes were confirmed to be ischemic by CT or MRI of the head. Index strokes were classified according to the prespecified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria and were blinded to genotype.

Genotyping

All samples were assayed with the Illumina Infinium Human-1 and HumanHap300 SNP chips (Illumina Inc). Details have been reported previously.10
Statistical Methods

Statistical analysis of the raw genotype data and moving window haplotype tests were done with the software SNPWA.

Using the case-control data, a series of generalized estimating equations were used that permitted inclusion of recognized stroke risk factors as covariates (age, sex, race, hypertension status, presence of atrial fibrillation, history of MI, smoking status, presence of diabetes mellitus, and family history of stroke).

For adjusted and unadjusted analyses, odds ratios in dominant, additive and recessive models and 95% CIs were computed.

Here we have focused on chr9p21 SNPs that were significantly associated with MI (rs10116277-rs1333040-rs2383207) in the work by Helgadottir and colleagues, and other SNPs from the same haplotype block (Figure).

Results

In unadjusted analyses, significant associations were observed for rs10116277, rs1333040, rs1333042, and rs2383207. The latter 3 SNPs showed an opposite association being protective against disease. Significant association was also seen between IS and almost all haplotypes involving these SNPs in additive and dominant models, before and after adjustment for stroke risk factors. See Tables 1 and 2.

Discussion

A significant association was seen between IS and SNPs initially associated with MI or located within the same LD block. This association was most striking at the haplotype level.

Our dataset is currently too small for us to determine whether one particular subtype of ischemic stroke contributes to this association or whether it is a general association with IS. It would be of interest to investigate this in a larger cohort, and such a study would also clarify if these variants may confer risk for IS independent of conventional risk factors. We are aware of the possibility of false-positive association;

### Table 1. Association Between SNPs at 9p21 and Risk for IS

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Unadjust.-A P Value OR (95% CI)</th>
<th>Unadjust.-D P Value OR (95% CI)</th>
<th>Unadjust.-R P Value OR (95% CI)</th>
<th>Adjusted-A P Value OR (95% CI)</th>
<th>Adjusted-D P Value OR (95% CI)</th>
<th>Adjusted-R P Value OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.309</td>
<td>0.699</td>
<td>0.038</td>
<td>0.133</td>
<td>0.102</td>
<td>0.415</td>
</tr>
<tr>
<td>rs10116277*</td>
<td>0.88</td>
<td>(0.94–1.58)</td>
<td>0.65</td>
<td>1.26</td>
<td>1.50</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>0.806</td>
<td>0.895</td>
<td>0.628</td>
<td>0.907</td>
<td>0.802</td>
<td>0.954</td>
</tr>
<tr>
<td>rs1547705</td>
<td>1.05</td>
<td>(0.71–1.54)</td>
<td>1.45</td>
<td>0.97</td>
<td>0.783</td>
<td>0.984</td>
</tr>
<tr>
<td>3</td>
<td>0.104</td>
<td>0.032</td>
<td>0.796</td>
<td>0.116</td>
<td>0.409</td>
<td>0.104</td>
</tr>
<tr>
<td>rs1333040*</td>
<td>1.23</td>
<td>(0.96–1.59)</td>
<td>1.06</td>
<td>0.782</td>
<td>0.781</td>
<td>0.695</td>
</tr>
<tr>
<td>4</td>
<td>0.247</td>
<td>0.042</td>
<td>0.872</td>
<td>0.077</td>
<td>0.386</td>
<td>0.046</td>
</tr>
<tr>
<td>rs1333042</td>
<td>1.15</td>
<td>(0.91–1.47)</td>
<td>0.97</td>
<td>0.765</td>
<td>0.803</td>
<td>0.615</td>
</tr>
<tr>
<td>5</td>
<td>0.153</td>
<td>0.042</td>
<td>0.806</td>
<td>0.079</td>
<td>0.323</td>
<td>0.065</td>
</tr>
<tr>
<td>rs2383207*</td>
<td>1.19</td>
<td>(0.94–1.52)</td>
<td>1.05</td>
<td>0.766</td>
<td>0.775</td>
<td>0.643</td>
</tr>
</tbody>
</table>

A indicates additive model; D, dominant model; R, recessive model; Unadjust., P value before adjusted for known stroke risk factors.

*SNPs previously associated with MI.

### Table 2. Association Between SNP Haplotypes at 9p21 and Risk for IS Focusing on Haplotypes That Contain SNPs Previously Associated With MI (numbers 1, 3 and 5)

<table>
<thead>
<tr>
<th>Data</th>
<th>SNP Range</th>
<th>Model</th>
<th>Calls</th>
<th>P Values</th>
<th>Odds Ratios</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>3–4–5</td>
<td>additive</td>
<td>CAA</td>
<td>0.0177</td>
<td>1.36</td>
<td>(1.06–1.76)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1–2–3–4–5</td>
<td>additive</td>
<td>GACAA</td>
<td>0.0196</td>
<td>1.36</td>
<td>(1.05–1.75)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>3–4–5</td>
<td>dominant</td>
<td>CAA</td>
<td>0.0088</td>
<td>1.61</td>
<td>(1.13–2.29)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1–2–3–4–5</td>
<td>dominant</td>
<td>GACAA</td>
<td>0.0102</td>
<td>1.59</td>
<td>(1.12–2.27)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>3–4–5</td>
<td>additive</td>
<td>CAA</td>
<td>0.0207</td>
<td>1.44</td>
<td>(1.06–1.96)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1–2–3–4–5</td>
<td>additive</td>
<td>GACAA</td>
<td>0.0229</td>
<td>1.43</td>
<td>(1.05–1.95)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>3–4–5</td>
<td>dominant</td>
<td>CAA</td>
<td>0.0131</td>
<td>1.72</td>
<td>(1.12–2.65)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1–2–3–4–5</td>
<td>dominant</td>
<td>GACAA</td>
<td>0.0152</td>
<td>1.70</td>
<td>(1.11–2.62)</td>
</tr>
</tbody>
</table>

1=rs10116277, 2=rs1547705, 3=rs1333040, 4=rs1333042, 5=rs2383207.
however, it is encouraging that independent studies find common genetic risk variants for different but related diseases. 

CDKN2A and CDKN2B, the two characterized genes closest to the risk loci, are well established as tumor suppressor genes, and recent data suggest that their expression levels increase markedly with aging in primate skin, human vasculature and rodent and human kidney12; although clearly the proximity of these genes to the risk locus makes them strong candidates for functional analysis, it should be noted that genetic variability can affect distal gene expression, and thus the observed association in these diseases may not reflect a biological effect on CDKN2A or CDKN2B.

These data are the first step in a comprehensive association study, and studies with larger sample sizes are necessary in order to define a consistent association. However, these data are important for 3 reasons: first, they suggest a genetic association for stroke; second, they suggest that this association shares pathogenic mechanisms with heart disease and diabetes; third, they illustrate that public release of data can facilitate rapid risk locus discovery.

Acknowledgments

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Disclosures

None.

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