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

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 association under a dominant model; two of these SNPs, rs1333040 and rs2383207, were previously associated with MI and all exist within the same LD block. After adjustment for stroke risk factors, the risk allele in the SNP rs1333042 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 OR (95% CI)</th>
<th>Unadjust.-D OR (95% CI)</th>
<th>Unadjust.-R OR (95% CI)</th>
<th>Adjusted-A OR (95% CI)</th>
<th>Adjusted-D OR (95% CI)</th>
<th>Adjusted-R OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs10116277*</td>
<td>0.88 (0.94–1.58)</td>
<td>0.65 (0.43–0.98)</td>
<td>1.26 (0.93–1.70)</td>
<td>1.50 (0.92–2.45)</td>
<td>1.22 (0.75–1.99)</td>
</tr>
<tr>
<td>2</td>
<td>rs1547705</td>
<td>1.05 (0.71–1.54)</td>
<td>1.45 (0.32–6.54)</td>
<td>0.97 (0.60–1.56)</td>
<td>0.783 (0.12–5.27)</td>
<td>0.984 (0.59–1.65)</td>
</tr>
<tr>
<td>3</td>
<td>rs1333040*</td>
<td>0.104 (0.96–1.59)</td>
<td>1.06 (1.03–2.15)</td>
<td>0.782 (0.58–1.06)</td>
<td>0.781 (0.43–1.40)</td>
<td>0.695 (0.45–1.08)</td>
</tr>
<tr>
<td>4</td>
<td>rs1333042</td>
<td>1.15 (0.91–1.47)</td>
<td>0.97 (1.01–2.25)</td>
<td>0.765 (0.65–1.45)</td>
<td>0.803 (0.57–1.03)</td>
<td>0.615 (0.38–0.99)</td>
</tr>
<tr>
<td>5</td>
<td>rs2383207*</td>
<td>1.19 (0.94–1.52)</td>
<td>1.05 (1.01–2.21)</td>
<td>0.766 (0.70–1.59)</td>
<td>0.775 (0.57–1.03)</td>
<td>0.643 (0.47–1.28)</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 kidney; 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.

References


Figure. Pairwise LD in the region 21.9 to 22.1 Mb (NCBI build 36.2) on chromosome 9 downloaded from the HapMap database (www.hapmap.org) for the CEU population. This plot shows SNPs assayed in the present study using Illumina Infinium Human-1 and HumanHap300 assays (numbers 1 to 5) and SNPs previously associated with MI. Numbers within the diamonds are $D'$ values (a measure of LD strength) for the respective SNP pairs. Solid red diamonds represent absolute LD ($D'=1$), blue diamonds represent strong LD with low level of significance. Numbers in gray within white diamonds represent a high probability or evidence of historical recombination. The figure shows clearly how one LD block comprising all SNPs, significantly associated with MI (squares) or IS (asterisks). WTCCC indicates Wellcome Trust Case Control Consortium.

3. Wellcome Trust Case Control C. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447:617–668.


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