A Whole-Genome Scan for Stroke or Myocardial Infarction in Family Blood Pressure Program Families

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Background and Purpose—Atherothrombotic diseases, including stroke and myocardial infarction, share a common pathogenesis. Chromosomal regions have been linked to atherothrombotic diseases in family studies, and association studies have identified candidate gene polymorphisms that affect the risk of stroke and/or myocardial infarction. Using data from the Family Blood Pressure Program, we tested for chromosomal regions linked to the composite phenotype of stroke or myocardial infarction in a large set of hypertensive families.

Methods—Nonparametric linkage analysis was implemented in MERLIN, which tests for excess allele-sharing among affected siblings. Empirical distributions based on gene dropping simulations were constructed for each test statistic, and the \(-\log_{10}\) of the associated probability values were compared.

Results—Analyses were based on 9607 individuals in 226 black, 395 Hispanic, and 207 white families; 106 families had multiple affected individuals. Several regions showed linkage to stroke or myocardial infarction, most significantly in Hispanics on chromosomes 2p21 (\(-\log_{10} P=3.0\)) and 7q21.1 (\(-\log_{10} P=2.8\)), 9q32 in blacks and Hispanics (\(-\log_{10} P=3.0\)), 11p13 in blacks (\(-\log_{10} P=2.1\)), and 12q24.33 in whites (\(-\log_{10} P=3.0\)).

Conclusions—There is statistically significant evidence for loci affecting stroke or myocardial infarction on chromosomes 2, 9, and 12. (Stroke. 2008;39:1115-1120.)

Key Words: cerebrovascular accident ■ epidemiology ■ linkage (genetics) ■ LOD score ■ myocardial infarction

Stroke and myocardial infarction (MI) are common manifestations of atherothrombotic disease and together are the leading cause of death and disability in the United States. There are approximately 865 000 new and recurrent MIs in the United States each year and 750 000 new and recurrent cases of stroke. Ischemic blockage accounts for approximately 80% of stroke events; approximately 10% are due to intracerebral hemorrhage; 10% result from subarachnoid hemorrhage.

Ischemic stroke and MI are similar phenotypes despite occurring in different locations. They share several pathophysiological mechanisms (eg, inflammation, atherosclerosis, plaque rupture, thrombosis, ischemic blockage) and risk factors (hypertension, smoking, dyslipidemia, no or high alcohol consumption, inflammation, homocystinemia, hypercoagulable states, diabetes). Both phenotypes have a substantial familial component. Monozygotic twins are 1.6 times more likely to be concordant for stroke than dizygotic twins; several studies show a small, significant increase in stroke risk in those with a family history of stroke. MIs are 2.48-fold more common in individuals with a sibling who has had an MI compared with those without. The heritability of arterial and venous thrombosis is estimated at 60% using liability threshold models. Past genome scans for stroke and MI have identified potential regions and genes of interest in various populations.

Combining stroke and MI allows detection of genes common to the pathogenesis of both and may increase power for detection of susceptibility loci. Therefore, we performed a genome scan for stroke or MI in black, Hispanic, and white families enrolled in the Family Blood Pressure Program (FBPP), the largest sample of families with data available for linkage analysis on stroke and MI. A combination of liability threshold and affected sibpair (ASP) models was used to detect linked regions.

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Materials and Methods

Recruitment and Participants
FBPP data were obtained by pooling data from 4 National Heart Lung and Blood Institute hypertension genetics studies, The Genetic Epidemiology Network (GenNet), the Genetic Epidemiology Network of Atherosclerosis (GENOA), the Hypertension Genetic Epidemiology Network (HyperGEN), and Stanford Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRE). Recruitment and inclusion criteria varied across networks and are published elsewhere.16 Informed consent was obtained from all participants.

Data Collection
All networks measured a standard set of 95 phenotypes. Stroke and MI data were obtained through self-report based on the following questions: “Have you ever been told by a doctor that you had a heart attack?” and “Have you ever been told by a doctor that you had a stroke or transient ischemic attack?” Diabetes status was also assessed in this manner. Lifestyle factors such as smoking status, alcohol use, and family structure were ascertained by interview. Anthropomorphic data were recorded according to standardized protocols.

Other Measurements
Systolic and diastolic blood pressures were measured using an automated device with a consistent protocol across networks. Blood pressure was measured 3 times on each participant and averaged for analysis. Lipids were measured spectrophotometrically using enzymatic methods (Cobas Mira analyzer; Roche Diagnostics) in SAPPHIRE, the ProAct cholesterol system (Roche) in GenNet, and an enzymatic method (COBAS GENOA, an enzymatic method in SAPPHIRE, the ProAct cholesterol system (Roche) in GenNet, and an enzymatic method (COBAS FARA centrifugal analyzer; Roche Diagnostics) in HyperGEN.

Genotyping
Genotyping was done by the National Heart, Lung and Blood Institute Mammalian Genotyping Service (Marshfield, Wis). Genotypes for 391 short tandem repeat polymorphisms were determined by automated polymerase chain reaction and scanning fluorescence detection.

Statistical Analysis
Race-specific and combined Kong-Cox logarithm of the odds (LOD) scores17 were calculated using the nonparametric LOD feature in MERLIN,18 which tests for excess allele sharing among an ASP. LOD scores were calculated within racial groups and summed to obtain whole-sample estimates. In MERLIN,18 empirical probability values were also calculated for Kong-Cox LOD scores based on gene-dropping simulations in which a single, fully informative marker is repeatedly, randomly “dropped” from parents to offspring in pedigrees with the same structure and affection status as the actual families. A LOD score was computed for each simulation (N=1 000 000), the set of which was considered as an empirical distribution under the null hypothesis of no linkage between marker and trait loci.

Results
The overall FBPP characterized 9607 individuals. There were 226 black, 395 Hispanic, and 207 white families; 880 individuals reported a history of stroke or MI and 83 individuals reported a history of both events. Table 1 shows baseline characteristics.

For ASP linkage analysis, there were 828 individuals in 103 families with multiply affected individuals: 29 families had a pair of MI-affected individuals and 10 families had a pair affected for stroke; 30 families had a discordantly affected pair (ie, one had a stroke, one had MI); 13 families had one member of the relative pair with both events and the other had one event. Of the 21 families with more than 2 affected members, 5 had all affected members concordant for a history of MI and 2 for history of stroke. The remaining 14 families had various combinations of discordant and discordant affection status among relative pairs. Kong-Cox LOD scores were computed for 32 black, 26 Hispanic, and 37 white families with an ASP. The Figure shows the Kong-Cox LOD plots for each race group and in the combined sample. Table 2 shows the −log10 of the empirically determined probability values greater than 2.0 and their chromosomal locations.

Discussion
In the FBPP, we found 3 significant linkage regions for the composite phenotype that are consistent with prior reports (summarized in Table 3). Although our LOD scores in these previously identified linkage regions do not achieve genomewide significance, nor were they our most significant results, we show −log10 probability values of 1.9, 1.9, and 1.0 at 2q36-q37.3, 2q21.1-22, and 14q32.2, respectively. The previous 2q36-q37.3 signal was for MI or coronary heart disease in a small sample of Australian ASPs,8 whereas our signal was mainly in blacks. Genes of interest in the vicinity include
insulin receptor substrate-1, the high-density lipoprotein cholesterol-binding protein, and calpain 10. Similarly, our modest signal at 14q32.2 was driven mainly by black and Hispanic families, whereas the original finding was in German families. Our replication of linkage on 2q21.1-22 was also observed in both black and Hispanic families, but not in whites. The original finding was in Finnish families ascertained for premature coronary heart disease. This region

Figure. Kong-Cox LOD plots for stroke or MI in affected sibpairs.
contains the gene thrombospondin type-1 domain-containing protein 7B precursor. The protein’s function has not been characterized, although thrombospondin type-1, in addition to its role in platelet aggregation and coagulation, interacts with platelet-derived growth factor to stimulate smooth muscle cell proliferation and with platelet glycoprotein IV (CD36) to inhibit angiogenesis. The latter findings are of particular interest because CD36, discussed in more detail subsequently, lies directly under our chromosome 7 peak. If the thrombospondin type-1 domain on this protein has similar functions, it could conceivably affect atherogenesis, stroke, and MI through multiple pathways.

In addition to the genes located in previously identified regions, our novel linkage regions also contain interesting candidates. One of these lies directly under the peak at 20 cM on chromosome 4. HS3ST1 encodes the rate-limiting enzyme heparan sulfate 3-O-sulfotransferase-1, which in turn controls production of the anticoagulant heparan sulfate (HSact) in endothelial cells. HajMohammadi et al created an HS3ST1 double knockout mouse strain; although their tissue HSact levels were dramatically reduced, the knockout mice showed a similar hemostatic profile as wild-type mice. The authors suggest that deficiency in the activity of the human HS3ST1 gene product might exert an affect only when combined with deficiencies for other HS3ST1 isozymes or other anticoagulants/fibrinolytics.

Table 2. Chromosomal Regions With $-\log_{10} P$ Values of Combined Race Kong-Cox LOD Scores Greater Than 2.0

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Location (cM)</th>
<th>$-\log_{10}$ Kong-Cox P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>243</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>2.2</td>
</tr>
<tr>
<td>7</td>
<td>94</td>
<td>2.8</td>
</tr>
<tr>
<td>9</td>
<td>99</td>
<td>3.0</td>
</tr>
<tr>
<td>11</td>
<td>45</td>
<td>2.1</td>
</tr>
<tr>
<td>12</td>
<td>166</td>
<td>3.0</td>
</tr>
<tr>
<td>20</td>
<td>97</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 3. Linkage Peaks From Previous Genome Scans for Atherothrombotic Disease End Points and $-\log_{10} P$ Values at Corresponding Locations in the Current Study

<table>
<thead>
<tr>
<th>Chromosomal Location</th>
<th>LOD Score</th>
<th>Phenotype</th>
<th>Population</th>
<th>$-\log_{10} P$ Value From Current Study (∆ location)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2q36-q37.3</td>
<td>2.63</td>
<td>MI/CHD</td>
<td>53 Australian sibpairs</td>
<td>1.9 (15 cM)</td>
</tr>
<tr>
<td>2q21.1-22</td>
<td>3.0</td>
<td>Premature CHD</td>
<td>156 Finnish families</td>
<td>1.9 (5 cM)</td>
</tr>
<tr>
<td>5q12</td>
<td>4.4</td>
<td>Ischemic stroke</td>
<td>476 Icelandic patients and 438 relatives</td>
<td>0.7</td>
</tr>
<tr>
<td>5q12</td>
<td>2.06</td>
<td>Ischemic stroke</td>
<td>56 Swedish families</td>
<td>0.7</td>
</tr>
<tr>
<td>10q23</td>
<td>2.06</td>
<td>CHD</td>
<td>99 Indo-Mauritian families</td>
<td>0.0</td>
</tr>
<tr>
<td>13q12-13</td>
<td>2.86</td>
<td>MI</td>
<td>Icelandic females</td>
<td>0.1</td>
</tr>
<tr>
<td>14q32.2</td>
<td>3.9</td>
<td>MI</td>
<td>513 western European families</td>
<td>1.0</td>
</tr>
<tr>
<td>16q13</td>
<td>3.06</td>
<td>CHD</td>
<td>99 Indo-Mauritian families</td>
<td>0.8</td>
</tr>
<tr>
<td>Xq23-26</td>
<td>2.46</td>
<td>Premature CHD</td>
<td>156 Finnish families</td>
<td>NA</td>
</tr>
</tbody>
</table>

$\Delta$ location indicates difference between location of previously reported LOD score and location of peak in the current study; difference equals zero if not listed.

CHD indicates coronary heart disease; NA, not applicable.
In conclusion, we presented results of a whole-genome scan for stroke or MI conducted in a large, ethnically diverse pool of families. We used 2 fundamentally different linkage models to show statistically significant evidence for loci affecting stroke or MI on chromosomes 2, 9, and 12.

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**Disclosures**
None.

**References**

我们确认了我们的结果使用了一种阈值模型，该模型在SOLAR中实现了模拟LOD评分，并且将比较不同阈值模型之间的洛格10概率值。存在证据表明阈值模型在所有位置识别LOD和Kong-Cox LOD评分。存在证据对于阈值模型的差异，而模拟全范围的洛格10 LOD评分的值在阈值和与对应位置的LOD分析在ASX分析中是0.44，表明了发现的证据已经好地与另一个独立的研究方法之间的联系分析。

**Study Limitations**

性状测量方法的限制不包括在本研究中。第一步，stroke和MI历史数据是由自报来源收集的并且没有被确认。也有人记录了stroke或MI的结果，但在不涉及这些因素的情况下，MI已经记录。在增加stroke类型的情况下，没有识别出特定的具有强烈影响的预测因子。知晓大约80%的stroke类型是具有临床症状的，任何基因单独到此类型会过于被代表。

结合stroke和MI事件可能或可能不是一种不完整性。尽管有充分的证据提示stroke和MI是通过相似的病理生理机制来影响的，因此可能会有显著的异质性，在基因机制中影响风险，某些风险因素有更强的影响于单个stroke或MI。例如，血清胆固醇似乎是一个更重要的MI风险因素，25表明其效应似乎是相反的stroke和MI。在血管因素和某些风险因素对stroke和MI的影响在生理学风险因素：TIAT研究中是一致的。 LOD评分从多重性-调整的阈值模型是相似的。LOD评分的相关性表明，这些因素中的某些预测因子对stroke和MI具有相同的效应。

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