Genetic Variation in Soluble Epoxide Hydrolase (EPHX2) Is Associated With an Increased Risk of Ischemic Stroke in White Europeans

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Background and Purpose—Genetic variation in the EPHX2 gene region has been reported to influence susceptibility to ischemic stroke in blacks. We assessed the role of this gene region in white Europeans and performed analyses with regard to stroke subtypes.

Methods—Twenty-six single nucleotide polymorphisms in the EPHX2 gene region were genotyped in 601 patients with ischemic stroke and 736 matched controls. Cases were subtyped according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system. Analyses were done on single markers and haplotypes using a sliding-window approach.

Results—Three single nucleotide polymorphisms showed associations with an increased risk for ischemic stroke (allelic models; all P≤0.01). One of them retained statistical significance after correction for multiple testing. Associations were observed with large-vessel stroke and stroke of undetermined etiology but not with other stroke subtypes.

Conclusions—Our findings confirm and extend previous studies suggesting that genetic variation in or near the EPHX2 gene contributes to the risk of ischemic stroke. This association seems to be mediated predominantly by large-vessel disease. (Stroke. 2008;39:1593-1596.)

Key Words: stroke ■ genetics ■ EPHX2

Soluble epoxide hydrolase (sEH) catalyzes the degradation of epoxyeicosatrienoic acids (EETs), which have numerous effects on the vascular system including vascular inflammation and atherosclerosis. Recent evidence suggests that variation within or near EPHX2, the gene encoding sEH, contributes to both coronary heart disease and stroke. Fornage et al reported associations between specific haplotypes in the EPHX2 gene region and risk of incident ischemic stroke (IS) in blacks. However, stroke subtypes were not considered and the results in white Americans were less clear. We set out to (1) evaluate the potential role of EPHX2 in a large group of white European IS patients and (2) explore relationships with specific etiologic subgroups.

Materials and Methods

The study population consisted of 601 white European patients (mean age 64±13.9 years, 377 men) recruited from a single dedicated Stroke Unit at the authors’ institution (Stroke Unit, Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-Universität, Munich). Brain imaging was performed in all patients, with the majority (475 patients, 79%) undergoing MRI including diffusion-weighted imaging. Diagnosis of IS was based on neurological symptoms in combination with a documented acute infarct on neuroimaging. Diagnostic workup included: ECG (100%), duplex sonography of the extracranial arteries (100%), transcranial ultrasound (>90%), MR angiography (40%), and transthoracic echocardiography (60%). Holter monitoring and transesophageal echocardiography were performed if indicated.

Patients were subtyped using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system (supplemental Table I, available online at http://stroke.ahajournals.org). Stroke etiologies were as follows: large vessel, n=186; cardiac embolism, n=143; small vessel, n=86; other etiology, n=45; undetermined, n=141. The control group consisted of 736 unrelated individuals (mean age 62±11.7 years, 447 men) selected from the population-based KORA sample. Controls were matched for age and gender and were free of a history of previous stroke or transient ischemic attack, no other exclusion criteria were used.

Selected single-nucleotide polymorphisms (SNPs; Figure) included all informative SNPs from the previous investigation and additional SNPs to depict all genetic variation with a minor allele frequency ≥0.05 and an r² >90% (source: www.hapmap.org). Genotyping was performed with MALDI-TOF MS technology. The genotyping error rate was 0.1%. A sliding window approach (2 and 3 markers) was used for haplotype analyses. Case-control analyses
were done using \( \chi^2 \) tests. We tested polymorphic SNPs for correlation with overall IS and TOAST subgroups while applying both genotypic and allelic models. To correct for multiple testing, the Westfall/Young Permutation Method was applied which accounts for the dependence structure between SNPs, phenotypes and models.7

**Results**

Allele and genotype frequencies for all SNPs were in Hardy-Weinberg equilibrium in both cases and controls. Pairwise linkage disequilibrium between SNPs in the \( \text{EPHX2} \) gene region is presented in supplemental Figure Ia and Ib.

**Overall Ischemic Stroke**

Single marker tests based on an allelic model revealed associations between the minor alleles of SNP 9, SNP 14 and SNP 23 and an increased risk for IS (Table). Following correction for testing of multiple SNPs, a significant association was found for SNP 23 with an odds ratio of 1.53 (\( P=0.02 \)). The same 3 SNPs showed significant uncorrected probability values when applying a genotypic model (supplemental Table II). Haplotype analyses also showed significant associations but did not increase significance beyond that observed by the analysis of single SNPs (supplemental Tables III and IV). Because of high linkage disequilibrium between significant SNPs (SNPs 9 and 14: \( D’=1.0, r^2=0.94 \); SNP 9 and 23: \( D’=0.98, r^2=0.94 \); SNP 14 and 23: \( D’=0.99, r^2=0.95 \)), a haplotype containing the associated alleles of these three SNPs can be found and the corresponding odds ratio is 1.514 (1.14 to 2.01) with a probability value of 0.003.

**Etiologic Subgroups**

The three SNPs showing associations in the overall, unscreened sample were related specifically with large-vessel stroke and stroke of undetermined etiology, but not with other subtypes. The odds ratios for these associations ranged from 1.51 to 1.73 (supplemental Table V). The haplotype containing the associated alleles of the three SNPs showed an odds ratio of 1.59 (1.06 to 2.37, \( P=0.022 \)) in the large-vessel subgroup and an odds ratio of 1.54 (0.96 to 2.41, \( P=0.062 \)) in the subgroup of patients with undetermined etiology.

**Discussion**

The observed associations between genetic variation in the \( \text{EPHX2} \) gene region and risk of IS in white Europeans confirm and extend the initial findings on incident IS in the ARIC study.3 Three SNPs showed significant associations with overall IS. SNP 23 displayed the strongest association, which remained significant after correction for multiple testing. The associated variants identified here differ from those reported in the initial study.3 Population differences in genetic background and different patterns of association between as yet unidentified high-risk alleles and marker...
alleles or haplotypes might explain the different associations observed in the 2 studies.

Extending the analyses by Fornage et al., we performed subgroup analyses according to stroke etiology. The SNPs linked with IS in the overall sample were associated with both large-vessel stroke and stroke of undetermined etiology but not with other subtypes. The association with large-vessel stroke is consistent with a role of $EPHX2$ in atherosclerosis, and with recent data showing that specific haplotypes in $EPHX2$ modify the risk of coronary artery disease in white Americans.2 The association with stroke of undetermined etiology might also relate to atherosclerosis because this category includes both patients with multiple competing etiologies and patients with isolated low-grade stenosis of an upstream artery.

SNP 14 and SNP 23 are located within introns (Figure) and their functional relevance remains unknown. In contrast, SNP 9 causes an amino acid change in the sEH protein (R287Q). The 287Q allele has previously been shown to reduce the activity of sEH to 25% to 58% of the wild-type enzyme in vitro8,9 and is thus expected to increase EET levels in vivo. Moreover, the 287Q variant has been demonstrated to enhance neuronal survival after ischemic injury in a cell culture model,10 and an sEH inhibitor has been shown to reduce infarct size in an experimental stroke model.11 Thus, a protective effect of the 287Q allele on stroke and cardiovascular disease might be expected. Yet, we found an association with increased risk for IS for the 287Q allele. This conflicts with the experimental data mentioned above8–11 but is in line with findings from the CARDIA study, which showed an increased risk for coronary artery calcification in blacks for the 287Q allele.6 Additional studies are needed to fully understand the effects of this variant in vivo. Alternatively, the 287Q allele might not be causative itself but instead represent a marker for an unidentified high-risk allele in linkage disequilibrium with this polymorphism.

A major strength of the present study was the use of a large, carefully phenotyped sample and rigorous control for testing of multiple SNPs which was not performed in the initial study.3 A potential limitation of our study is the lack of a correction for vascular risk factors, which was not possible because of incomplete information in some subjects. Part of the study population was phenotyped retrospectively reviewing all available information (discharge reports, patient files, CT and MR images, results of laboratory tests, etc) and in those early cases, absence of risk factors was not explicitly documented. Therefore, we considered the information on risk factors to be not sufficiently reliable and decided not to use it for a multivariate analysis. Future studies may consider

### Table. Ischemic Stroke Overall (n=601 patients), Allelic Model

<table>
<thead>
<tr>
<th>SNP- No.</th>
<th>SNP-ID</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>$P$ Value (nominal)</th>
<th>$P$ Value (WY)</th>
<th>MAF (cases)</th>
<th>MAF (controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>. . .</td>
<td>0.812</td>
<td>0.627–1.053</td>
<td>0.116</td>
<td>0.656</td>
<td>0.097</td>
<td>0.119</td>
</tr>
<tr>
<td>2</td>
<td>rs17057255</td>
<td>1.213</td>
<td>0.390–3.771</td>
<td>0.738</td>
<td>1.000</td>
<td>0.005</td>
<td>0.004</td>
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<td>3</td>
<td>rs7846038</td>
<td>0.828</td>
<td>0.638–1.075</td>
<td>0.156</td>
<td>0.775</td>
<td>0.097</td>
<td>0.117</td>
</tr>
<tr>
<td>5</td>
<td>rs2741334</td>
<td>0.781</td>
<td>0.583–1.046</td>
<td>0.096</td>
<td>0.601</td>
<td>0.072</td>
<td>0.092</td>
</tr>
<tr>
<td>6</td>
<td>rs17057284</td>
<td>0.843</td>
<td>0.649–1.094</td>
<td>0.199</td>
<td>0.859</td>
<td>0.097</td>
<td>0.115</td>
</tr>
<tr>
<td>7</td>
<td>rs13262930</td>
<td>1.094</td>
<td>0.917–1.306</td>
<td>0.320</td>
<td>0.962</td>
<td>0.343</td>
<td>0.314</td>
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<tr>
<td>8</td>
<td>rs7816586</td>
<td>0.981</td>
<td>0.841–1.144</td>
<td>0.804</td>
<td>1.000</td>
<td>0.211</td>
<td>0.196</td>
</tr>
<tr>
<td>9</td>
<td>rs751141</td>
<td>1.458</td>
<td>1.117–1.902</td>
<td>0.005*</td>
<td>0.055</td>
<td>0.121</td>
<td>0.083</td>
</tr>
<tr>
<td>10</td>
<td>rs4149243</td>
<td>0.916</td>
<td>0.741–1.132</td>
<td>0.416</td>
<td>0.990</td>
<td>0.175</td>
<td>0.191</td>
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<td>11</td>
<td>rs721619</td>
<td>0.925</td>
<td>0.785–1.090</td>
<td>0.351</td>
<td>0.976</td>
<td>0.450</td>
<td>0.486</td>
</tr>
<tr>
<td>12</td>
<td>rs4149246</td>
<td>1.009</td>
<td>0.719–1.415</td>
<td>0.959</td>
<td>1.000</td>
<td>0.057</td>
<td>0.057</td>
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<tr>
<td>13</td>
<td>rs4149247</td>
<td>0.895</td>
<td>0.723–1.108</td>
<td>0.308</td>
<td>0.956</td>
<td>0.168</td>
<td>0.188</td>
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<tr>
<td>14</td>
<td>rs7357432</td>
<td>1.463</td>
<td>1.116–1.917</td>
<td>0.006*</td>
<td>0.059</td>
<td>0.116</td>
<td>0.079</td>
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<td>15</td>
<td>rs10503812</td>
<td>0.918</td>
<td>0.740–1.140</td>
<td>0.439</td>
<td>0.994</td>
<td>0.168</td>
<td>0.183</td>
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<td>16</td>
<td>rs12547188</td>
<td>0.891</td>
<td>0.629–1.262</td>
<td>0.516</td>
<td>0.998</td>
<td>0.051</td>
<td>0.057</td>
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<tr>
<td>17</td>
<td>rs2234887</td>
<td>0.975</td>
<td>0.714–1.331</td>
<td>0.872</td>
<td>1.000</td>
<td>0.068</td>
<td>0.069</td>
</tr>
<tr>
<td>18</td>
<td>rs4149252</td>
<td>0.894</td>
<td>0.720–1.110</td>
<td>0.310</td>
<td>0.957</td>
<td>0.164</td>
<td>0.184</td>
</tr>
<tr>
<td>19</td>
<td>rs4149253</td>
<td>0.953</td>
<td>0.679–1.338</td>
<td>0.781</td>
<td>1.000</td>
<td>0.055</td>
<td>0.058</td>
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<td>rs2071575</td>
<td>1.117</td>
<td>0.939–1.330</td>
<td>0.212</td>
<td>0.877</td>
<td>0.369</td>
<td>0.330</td>
</tr>
<tr>
<td>22</td>
<td>rs13269963</td>
<td>1.177</td>
<td>0.965–1.436</td>
<td>0.108</td>
<td>0.637</td>
<td>0.239</td>
<td>0.203</td>
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<tr>
<td>23</td>
<td>rs2291635</td>
<td>1.532</td>
<td>1.172–2.003</td>
<td>0.002*</td>
<td>0.020*</td>
<td>0.121</td>
<td>0.079</td>
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<td>24</td>
<td>rs1126452</td>
<td>1.131</td>
<td>0.945–1.353</td>
<td>0.180</td>
<td>0.832</td>
<td>0.332</td>
<td>0.293</td>
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<tr>
<td>25</td>
<td>rs1042032</td>
<td>1.129</td>
<td>0.944–1.351</td>
<td>0.184</td>
<td>0.837</td>
<td>0.333</td>
<td>0.295</td>
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<td>26</td>
<td>rs4149259</td>
<td>0.903</td>
<td>0.728–1.120</td>
<td>0.351</td>
<td>0.976</td>
<td>0.165</td>
<td>0.183</td>
</tr>
</tbody>
</table>

WY indicates corrected for testing multiple SNPs using Westfall and Young algorithm.

*Significant ($P<0.05$).

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gene-environment interactions including an account of cigarette smoking.  

Acknowledgments

The authors thank all subjects who participated in this study and Michael O’Sullivan for critically reading the manuscript.

Sources of Funding

H.-E. Wichmann was supported by the German Ministry of Education and Research through the National Genome Research Network (NGFN): 01GR0464, 01GR0411, 01GS0499. The KORA research platform was initiated and financed by the GSF–National Research Center for Environment and Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria.

Disclosures

None.

References


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*Stroke*. 2008;39:1593-1596; originally published online March 6, 2008; doi: 10.1161/STROKEAHA.107.502179

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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