Promoter Polymorphisms in the Nitric Oxide Synthase 3 Gene Are Associated With Ischemic Stroke Susceptibility in Young Black Women

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Background and Purpose—Endothelial nitric oxide exerts a variety of protective effects on endothelial cells and blood vessels, and therefore the nitric oxide synthase 3 gene (NOS3) is a logical candidate gene for stroke susceptibility.

Methods—We used the population-based Stroke Prevention in Young Women case-control study to assess the association of five NOS3 polymorphisms in 110 cases (46% black) with ischemic stroke and 206 controls (38% black), 15 to 44 years of age. Polymorphisms included 3 single nucleotide polymorphisms (SNPs) in the promoter region (−1468 T>A, −922 G>A, −786 T>C), 1 SNP in exon 7 (G894T), and 1 insertion/deletion polymorphism within intron 4.

Results—Significant associations with both the −922 G>A and −786 T>C SNPs with ischemic stroke were observed in the black, but not the white, population. This association was attributable to an increased prevalence of the −922 A allele (OR=3.0, 95% CI=1.3 to 6.8; P=0.005) and the −786 T allele (OR=2.9, 95% CI=1.3 to 6.4; P=0.005) in cases versus controls. These 2 SNPs were in strong linkage disequilibrium (D’=1.0), making it impossible to determine, within the confines of this genetic study, whether 1 or both of these polymorphisms are functionally related to NOS3 expression. Two sets of haplotypes were also identified, 1 of which may confer an increased susceptibility to stroke in blacks, whereas the other appears to be protective.

Conclusion—Promoter variants in NOS3 may be associated with ischemic stroke susceptibility among young black women. (Stroke. 2005;36:1848-1853.)

Key Words: genetics ■ nitric oxide ■ women and minorities ■ young, stroke in

Familial aggregation and twin studies provide evidence for a strong genetic component to ischemic stroke risk, particularly for early-onset cases.1-5 Endothelial nitric oxide synthase (NOS3) is a logical candidate gene because of its ability to generate NO, which plays a central role in the maintenance of vascular homeostasis, including regulation of the cerebral circulation. NO is also a potent vasodilator7 and inhibits platelet aggregation.8 Impaired endothelial-mediated vasodilation is a common feature of many vascular risk factors, and experimental evidence strongly supports a role for impaired NO-dependent vasomotor reactivity in the pathophysiology of stroke.9,10

Association Studies with NOS3 and ischemic stroke have been conducted exclusively in older populations and typically included only 1 or a few SNPs genotyped in various ethnic groups.11-13 These studies have yielded conflicting results. To further characterize the role of NOS3 in ischemic stroke, we evaluated 5 polymorphisms among black and white cases and controls in the Stroke Prevention in Young Women Study (SPYW).

Materials and Methods

Population

The SPYW study is a population-based, case-control study initiated to examine risk factors for ischemic stroke. Both cases and their comparison group (controls) were identified from a study area that included all of Maryland except the far Western panhandle, Washington DC, and southern portions of both Pennsylvania and Delaware. Cases and controls were recruited between February 26, 1992 and January 1, 1996. Institutional review committees at collaborating institutions approved the study and participants gave informed consent.

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The baseline characteristics of the SPYW population are shown in Table 1. Cases (51 black, 59 white) were women 15 to 44 years of age with a first cerebral infarction, who were identified by discharge surveillance at all 59 hospitals in the study area or through direct referral by regional neurologists. All stroke cases were adjudicated by 2 neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been described previously. Recruitment within 1 year of stroke was required for participation. Controls (78 black, 128 white) were identified by random-digit dialing and included women without a history of stroke, frequency matched by age and geographic region of residence to the cases.

Hypertension, diabetes mellitus, and angina or myocardial infarction were determined by asking the participant (or her proxy when the participant was unable to answer) whether she had ever been told. Current smoking status were determined by participant or proxy referral by regional neurologists. All stroke cases were adjudicated by 2 neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been described previously. Recruitment within 1 year of stroke was required for participation. Controls (78 black, 128 white) were identified by random-digit dialing and included women without a history of stroke, frequency matched by age and geographic region of residence to the cases.

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TABLE 3. Analysis of Stroke Susceptibility and NOS3 Genotypes

<table>
<thead>
<tr>
<th>Haplotype No.</th>
<th>Haplotype</th>
<th>White Cases (n=118)</th>
<th>White Controls (n=254)</th>
<th>Black Cases (n=102)</th>
<th>Black Controls (n=158)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AATcG</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>AATbG</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AATbG</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>AATcG</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AGCaG</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
<td>0.08*</td>
</tr>
<tr>
<td>6</td>
<td>AGCaT</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>AGGbG</td>
<td>0.06</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>AGGbT</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>TATcG</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>TATG</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>TATcG</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>TATbT</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>TATcG</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>TATcG</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n indicates number of haplotypes. *P=0.001.
**NOS3** variants stratified by stroke subtypes and limited the power for detecting interactions with other risk factors. This study also has several strengths. First, we examined multiple polymorphisms, as well as estimated haplotypes, in our population, allowing for a more comprehensive evaluation of the gene. Second, our study is the first to examine the association of **NOS3** variants with stroke in blacks. Third, this study involved the use of a young stroke population, which may have enhanced our ability to detect a genetic contribution to risk.

In conclusion, our study extends prior work showing the association of **NOS3** promoter variants with isolated lacunar stroke in an elderly white population to early onset ischemic stroke of diverse subsets among blacks. More comprehensive studies of the association between **NOS3** genetic variation and both functional correlates and stroke risk in larger, ethnically diverse populations are needed.

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**References**

Are We in Another Unavoidable ‘Diagnose and Adios’ Era?

None of us in stroke management regret the passage of the “diagnose and adios” era, humorously criticized earlier. We know well that opportunities for stroke management are arising for more definitive studies. A revolution in imaging, the rush to apply results from clinical trials, and attempts to shorten the time frame for action are daily practice because neurologists are interventionists similar to their colleagues in cardiology and emergency medicine. However, with the pace of accumulated data from leisurely approached genetic association studies, we confront the issue of hereditary risk factors.

In this issue of Stroke, Howard et al report on a well-organized, population-based study in 15- to 44-year-old women. Among the 5 nitric oxide synthase (NOS)3 polymorphisms in 110 cases (46% black) with ischemic stroke and 206 controls (38% black) who were recruited over 4 years, these investigators found that the −922 G/A and −786 T/C polymorphisms may be associated with ischemic stroke susceptibility among young black women. Although the small sample size precluded extensive analysis, this study highlights the importance of genetic studies of stroke at a young age in different ethnic groups.

Genetic factors may play a role in cerebral vascular disease and may act via endothelial dysfunction, predominantly in the young. NO synthesized by endothelial NOS is a key mediator of endothelial function, and it could be a candidate gene for stroke. The NOS3 intron 4ab insertion/deletion genotype, but not the −786 T/C or the G894T genotype, was reported to be associated with isolated lacunar infarction in an English study but not a Japanese study. The NOS3 exon 7 polymorphism (G894T) was reported to be not associated with stroke and transient ischemic attack or the degree of carotid stenosis in patients with cerebrovascular disease. The aforementioned results show that the association between the NOS gene and ischemic stroke is controversial and that there is a possible positive association between the NOS3 intron 4ab insertion/deletion genotype and small-vessel disease in white populations or a possible negative association in other ethnicities.

It is known that the etiology of ischemic stroke in the young is heterogeneous and diverse and that there might be racial differences. Previous reports regarding ischemic stroke in the young showed that blacks, unlike whites, more commonly had the stroke subtype of small-vessel occlusion than large-artery atherosclerosis and that strokes due to other determined and undetermined causes occurred in more than half of young stroke patients in most studies. The finding that the −922 G/A and −786 T/C polymorphisms may be associated with ischemic stroke susceptibility among young black women should be confirmed in a large, international, cooperative study. The population-based study design of Howard et al yielded important information, but the pheno-
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References

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