Genetic Evidence That Nitric Oxide Modulates Homocysteine

The NOS3 894TT Genotype Is a Risk Factor for Hyperhomocystenemia

Karen S. Brown, Leo A.J. Kluijtmans, Ian S. Young, Jayne Woodside, John W.G. Yarnell, Dorothy McMaster, Liam Murray, Alun E. Evans, Colin A. Boreham, Helene McNulty, J.J. Strain, Laura E. Mitchell, Alexander S. Whitehead

Objective—Mild hyperhomocysteine is an independent, graded risk factor for cardiovascular disease. Genetic determinants of hyperhomocysteine include functional polymorphisms in several folate/homocysteine metabolic enzymes. Nitric oxide may also modulate plasma homocysteine (tHcy) concentrations, either by direct inhibition of methionine synthase or via an indirect effect on folate catabolism.

Methods and Results—The hypothesis that the endothelial nitric oxide synthase (NOS3) G894T polymorphism is a genetic determinant of tHcy concentrations was tested in 2 independent healthy adult populations. In both populations, NOS3 genotype was significantly associated with tHcy concentrations in nonsmokers with low folate (P ≤ 0.03 for each). Models were constructed to adjust for known determinants of tHcy concentrations and test for interactions between NOS3 genotype and these determinants in nonsmokers from each population. NOS3 genotype remained a significant determinant of tHcy concentrations after adjustment. Interactions between NOS3 genotype and serum folate were significant in both populations, and the interaction between NOS3 genotype and MTHFR C677T genotype was significant in the larger population.

Conclusions—These data indicate that the NOS3 894TT genotype is a risk factor for elevated tHcy in healthy nonsmoking adults with low serum folate and supports the hypothesis that nitric oxide modulates homocysteine through an effect on folate catabolism. (Arterioscler Thromb Vasc Biol. 2003;23:1014-1020.)

Key Words: nitric oxide • nitric oxide synthase • hyperhomocystenemia • 5,10-methylenetetrahydrofolate reductase • smoking
the posttranslational level, is responsible for vascular NO production. NO, an important mediator of vascular muscle tone, possesses both antithrombotic and antioxidant properties. A decline in NO production and/or bioavailability in the vascular endothelium occurs early in the development of atherosclerosis and may play an etiologic role. It is well established that smoking, a strong risk factor for CVD as well as hyperhomocysteinemia, compromises NOS3 activity and is associated with impaired NO-dependent vasodilation.

Several polymorphisms have been identified in the NOS3 gene; however, only 1, G894T, mandates an amino acid change (Glu298Asp). The functional consequences of this polymorphism have yet to be determined, and its role in CVD remains unclear. Although some studies have shown positive associations with CVD, others have not.

Taken together, the published reports of modulation of homocysteine metabolism by NO and the possible association of the NOS3 G894T polymorphism with vascular diseases led us to hypothesize that G894T genotype may be a genetic determinant of tHcy concentrations. Based on the impact of smoking on NOS3 activity, we additionally hypothesized that smoking status may modify any such relationship between NOS3 genotype and tHcy. The data presented here strongly support the above hypotheses as well as the hypothesis that NO modulates tHcy concentrations indirectly through an effect on folate catabolism.

Methods

Subjects

Subjects from 2 independent, previously described study populations were included. Institutional review boards approved both studies, and all subjects provided written informed consent before participation.

The first population (Young Hearts) was drawn from the Young Hearts Project, an ongoing prospective study designed to assess the prevalence of coronary risk factors over time in young subjects from Northern Ireland. Biochemical data used for the analyses reported here were acquired at the third screening visit, at which time subjects were between 20 and 26 years old. Current smoking status was also determined at this visit.

Data from a second population (Industrial Workers), comprised of men aged 29 to 53 years employed by a single industrial company in Belfast, Northern Ireland, were analyzed to determine if the results obtained from the first population could be replicated. Subjects with diabetes and those who consumed nutritional supplements were excluded. Current smoking status was determined at the time of blood collection for genetic and biochemical analyses.

Determination of Homocysteine, Folate, and Vitamin B12

Blood samples were collected from fasting subjects for DNA extraction and determination of biochemical parameters. tHcy concentrations were determined previously using an established high-performance liquid chromatography method. Differences in the preparation of tHcy standards resulted in different absolute tHcy values between the 2 study populations. Serum folate and vitamin B12 concentrations were determined previously by time-resolved immunofluorescence on an AutoDelfia analyzer (Wallac) for the Young Hearts population and by a commercial kit (ICN Pharmaceuticals) for the Industrial Workers population.

Genetic Analysis

A heteroduplex generator (HG) method was developed for the NOS3 G894T genotyping. Briefly, a HG that spans the NOS3 genomic DNA region containing the G894T polymorphism was constructed. Its sequence is identical to that of the G allele, except for a 3-bp microdeletion introduced 16 bp 5’ of the polymorphic site and a 4-bp insertion (AAAA) added immediately 3’ to the polymorphic site. This HG is coamplified with genomic DNA by polymerase chain reaction (PCR). Denaturation and subsequent reannealing of the PCR products results in the formation of homoduplexes of genomic DNA, homoduplexes of HG, and heteroduplexes between genomic DNA and HG. The migration of the heteroduplexes in polyacrylamide gels is different from that of the homoduplexes and is determined by the size and sequence of the protruding loops in the regions of mismatch. The 3 G894T genotypes yield distinct, unambiguous migration patterns using this method (Figure 2).

Figure 1. Folate metabolic pathway. CBS indicates cystathionine β-synthase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxynucleoside monophosphate; dUMP, deoxyuridine monophosphate; HCY, homocysteine; MTHFR, 5,10-methylene tetrahydrofolate reductase; MTHFR, methionine synthase reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate; and TS, thymidylate synthase.
B12 quartile. All tests were 2-tailed, and values of $P \leq 0.05$ (or adjusted probability values, when appropriate) were considered statistically significant.

Generalized linear models were constructed to determine whether the observed associations between tHcy and NOS3 genotype were independent of other factors known to influence tHcy. The likelihood ratio test was used to determine the significance of the NOS3 genotype after adjusting for the effects of additional variables. A series of models were fitted to the data, as described in the supplemental methods. Serum folate, vitamin B12, age, and sex (Young Hearts only) were included in the models, irrespective of whether they were significantly related to tHcy concentrations. Models were constructed in a stepwise manner, starting with a model that contained NOS3 G894T genotype, serum folate, vitamin B12, age, and, for the Young Hearts population, sex. Interactions between NOS3 genotype and folate, vitamin B12, age, and sex, independent effects of known genetic determinants of tHcy (MTHFR C677T, MTR A2756G, and MTRR A66G genotypes), and interactions between NOS3 G894T genotype and other genotypes with significant independent effects were considered. Final models with and without terms containing NOS3 genotype were compared by likelihood ratio test to evaluate whether the terms contributed significantly to the overall model. The total amount of variation in tHcy explained by the final model was estimated by Efron’s pseudo-$R^2$. Additional details of the modeling process are provided in the online supplementary statistical methods, available at http://atvb.ahajournals.org. All statistical analyses were carried out using SAS version 8.2.

**Results**

The Young Hearts population was studied for an association between NOS3 G894T genotype and tHcy; genotypes for 357 subjects (186 male, 170 female, and 1 subject whose sex was not recorded) were determined. To determine if the results obtained in the Young Hearts population could be replicated, the relationship between the NOS3 G894T polymorphism and tHcy was studied in a second population, Industrial Workers; genotypes for 565 subjects were determined. All analyses were carried out separately in each population.

**Population Characteristics**

NOS3 G894T genotype frequencies, tHcy, and folate concentrations are summarized for each population as a whole and after stratification by smoking status in Table 1. The genotype distributions for each population overall and for the non-smoker and smoker subsets were not significantly different from those expected under Hardy-Weinberg equilibrium (data not shown) and are similar to those reported for other white populations.21,22

**Associations Between NOS3 G894T Genotype and Biochemical Parameters**

The NOS3 G894T genotypes were not significantly associated with tHcy in either population (Table 2). However, in both populations, those with the NOS3 894TT genotype tended to have higher tHcy concentrations than their 894GT and 894GG peers. NOS3 G894T genotype was not associated with folate concentration in either population.

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**TABLE 1. Population Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Nonsmokers</th>
<th>Smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Hearts Population, n (%)</td>
<td>357 (100)</td>
<td>216 (60.5)</td>
<td>138 (38.7)</td>
</tr>
<tr>
<td>NOS3 G894T genotype, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>150 (42.0)</td>
<td>91 (42.1)</td>
<td>58 (42.0)</td>
</tr>
<tr>
<td>GT</td>
<td>173 (48.5)</td>
<td>110 (50.9)</td>
<td>62 (44.9)</td>
</tr>
<tr>
<td>TT</td>
<td>34 (9.5)</td>
<td>15 (6.9)</td>
<td>18 (13.0)</td>
</tr>
<tr>
<td>Homocysteine,* $\mu$mol/L</td>
<td>8.9 (4.2 to 44.6)</td>
<td>8.6 (4.4 to 34.0)</td>
<td>9.7 (4.2 to 44.6)</td>
</tr>
<tr>
<td>Folate,* nmol/L</td>
<td>12.7 (3.2 to 213)</td>
<td>14.0 (3.2 to 213)</td>
<td>10.8 (4.9 to 45.0)</td>
</tr>
<tr>
<td>Industrial Workers Population, n (%)</td>
<td>565 (100)</td>
<td>421 (74.5)</td>
<td>141 (25.0)</td>
</tr>
<tr>
<td>NOS3 G894T genotype, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>254 (44.9)</td>
<td>180 (42.8)</td>
<td>74 (52.5)</td>
</tr>
<tr>
<td>GT</td>
<td>244 (43.2)</td>
<td>187 (44.4)</td>
<td>54 (38.3)</td>
</tr>
<tr>
<td>TT</td>
<td>67 (11.9)</td>
<td>54 (12.8)</td>
<td>13 (9.2)</td>
</tr>
<tr>
<td>Homocysteine,* $\mu$mol/L</td>
<td>7.1 (1.6 to 58.5)</td>
<td>7.0 (1.6 to 58.5)</td>
<td>7.4 (2.8 to 16.9)</td>
</tr>
<tr>
<td>Folate,* nmol/L</td>
<td>10.9 (3.4 to 45.3)</td>
<td>11.1 (3.4 to 45.3)</td>
<td>10.4 (3.4 to 33.5)</td>
</tr>
</tbody>
</table>

*Median (range).
Because NOS3 activity is compromised in smokers, NOS3 G894T genotype might be expected to be a significant determinant of tHcy concentrations only in nonsmokers. Therefore, each population was stratified by smoking status. Among nonsmokers, 894TT homozygotes had the highest median tHcy. However, the test for association of genotype with tHcy concentration did not reach significance for either population (\( P = 0.21 \) for Young Hearts and \( P = 0.09 \) for Industrial Workers, Table 2).

NOS3 genotype had no effect on median tHcy in smokers in either population (\( P = 0.88 \) for Young Hearts and \( P = 0.51 \) for Industrial Workers, Table 2). NOS3 G894T genotype may modulate tHcy concentrations indirectly through an effect on folate catabolism. If so, the impact of genotype would likely be most apparent in subjects with low folate status. Therefore, the relationship between the NOS3 G894T polymorphism and tHcy was evaluated in subjects with low serum folate (folate quartile 1). In both populations, genotype was significantly associated with tHcy concentration in subjects with a low folate phenotype (\( P = 0.02 \) for Young Hearts and \( P = 0.05 \) for Industrial Workers, Table 3). Again, 894TT homozygotes had the highest concentrations. NOS3 genotype was not associated with tHcy concentration in folate quartiles 2 to 4 of either population (data not shown).

When subjects were stratified by both folate and smoking status, NOS3 genotype was associated with tHcy concentrations only in nonsmokers from folate quartile 1 (\( P = 0.03 \) for both populations, Table 3). However, pairwise differences

### TABLE 2. Associations Between NOS3 G894T Genotype and Biochemical Parameters

<table>
<thead>
<tr>
<th>Population</th>
<th>Biochemical Parameter</th>
<th>GG</th>
<th>GT</th>
<th>TT</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>8.6 (4.2 to 37.4) 150</td>
<td>9.3 (4.4 to 41.0) 173</td>
<td>9.8 (5.3 to 44.6) 34</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Homocysteine, ( \mu )mol/L</td>
<td>8.2 (5.1 to 33.4) 91</td>
<td>9.0 (4.4 to 24.7) 110</td>
<td>9.4 (6.0 to 34.0) 15</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.7 (4.2 to 37.4) 58</td>
<td>9.6 (4.6 to 32.0) 62</td>
<td>9.8 (5.3 to 44.6) 18</td>
<td>0.88</td>
</tr>
<tr>
<td>Young Hearts</td>
<td>Homocysteine, ( \mu )mol/L</td>
<td>12.4 (3.2 to 213) 126</td>
<td>13.2 (3.9 to 53.2) 143</td>
<td>11.1 (5.6 to 39.6) 31</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.4 (3.2 to 213) 78</td>
<td>13.4 (3.9 to 53.2) 91</td>
<td>12.3 (5.8 to 21.5) 14</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.4 (4.9 to 45.0) 47</td>
<td>12.1 (5.1 to 41.1) 51</td>
<td>10.5 (5.6 to 39.6) 16</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Folate, ( \mu )mol/L</td>
<td>7.1 (2.7 to 58.5) 254</td>
<td>7.0 (3.5 to 24.3) 244</td>
<td>7.5 (1.6 to 27.4) 67</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.0 (2.7 to 58.5) 180</td>
<td>6.9 (3.5 to 24.3) 187</td>
<td>7.6 (1.6 to 27.4) 54</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.4 (2.8 to 16.9) 74</td>
<td>7.2 (4.0 to 15.3) 54</td>
<td>6.8 (4.6 to 9.9) 13</td>
<td>0.51</td>
</tr>
<tr>
<td>Industrial Workers</td>
<td>Homocysteine, ( \mu )mol/L</td>
<td>10.7 (3.4 to 41.7) 248</td>
<td>10.9 (3.4 to 45.3) 238</td>
<td>10.9 (4.1 to 33.5) 67</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.7 (3.4 to 41.7) 176</td>
<td>11.1 (3.6 to 45.3) 183</td>
<td>11.0 (5.0 to 33.1) 54</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.4 (4.8 to 23.8) 72</td>
<td>9.9 (3.4 to 28.6) 52</td>
<td>10.4 (4.1 to 33.5) 13</td>
<td>0.96</td>
</tr>
</tbody>
</table>

\( ^* \)Kruskal-Wallis Test. 
\( ^\dagger \)Median (range) \( n \). 

### TABLE 3. Associations Between NOS3 G894T Genotype and Homocysteine in Subjects From Serum Folate Quartile 1

<table>
<thead>
<tr>
<th>NOS3 G894T Genotype</th>
<th>GG</th>
<th>GT</th>
<th>TT</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Hearts Population( ^\dagger )</td>
<td>10.1 (5.1 to 37.4) 33</td>
<td>13.2 (8.4 to 41.0) 34</td>
<td>12.1 (8.1 to 44.6) 8</td>
<td>0.02§</td>
</tr>
<tr>
<td>All subjects</td>
<td>8.8 (5.1 to 33.4) 19</td>
<td>11.8 (8.4 to 21.0) 16</td>
<td>18.8 (12.7 to 34.0) 3</td>
<td>0.03§</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>11.5 (6.5 to 37.4) 14</td>
<td>14.4 (8.5 to 29.6) 17</td>
<td>11.1 (8.1 to 44.6) 5</td>
<td>0.24</td>
</tr>
<tr>
<td>Smokers</td>
<td>8.8 (5.0 to 58.5) 59</td>
<td>7.9 (4.1 to 24.3) 55</td>
<td>9.9 (6.8 to 27.4) 16</td>
<td>0.05§</td>
</tr>
<tr>
<td>Industrial Workers Population( ^\dagger )</td>
<td>8.8 (5.3 to 58.5) 39</td>
<td>8.0 (4.1 to 24.3) 37</td>
<td>10.3 (7.2 to 27.4) 12</td>
<td>0.03§</td>
</tr>
<tr>
<td>All subjects</td>
<td>8.2 (5.0 to 16.9) 20</td>
<td>7.8 (4.1 to 15.3) 16</td>
<td>8.4 (6.8 to 9.9) 4</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Homocysteine units: \( \mu \)mol/L.

\( ^* \)Kruskal-Wallis test. 
\( ^\dagger \)Median (range) \( n \). 
\( ^\ddagger \)Pairwise comparisons not significant after correction for multiple comparisons. 
\( ^\S \)\( P < 0.05 \) for GG vs GT.
between genotypes did not reach significance after correction for multiple comparisons for either population. NOS3 genotype was not associated with tHcy concentrations in smokers in folate quartile 1 (Table 3) or in quartiles 2 to 4 (data not shown) for either population.

It is postulated that NO inhibits MTR by oxidizing its vitamin B_{12} cofactor to an inactive state. However, after stratification of subjects according to vitamin B_{12} status, no significant associations between genotype and tHcy concentration were observed in the low vitamin B_{12} quartile in either population (data not shown).

To determine whether our findings remained significant after correction for factors known to influence tHcy, we next constructed generalized linear models for nonsmokers in each population; final models are given in Table 4. Our analyses indicate that in the Young Hearts population, NOS3 G894T genotype, MTHFR C677T genotype, folate, and vitamin B_{12}, as well as an interaction term between NOS3 genotype and folate, are important determinants of tHcy. In the Industrial Workers population, important determinants were NOS3 C677T genotype, MTHFR C677T genotype, MTR A2756G genotype, MTRR A66G genotype, folate, and vitamin B_{12}, as well as interaction terms between NOS3 genotype and folate and between NOS3 genotype and MTHFR C677T genotype.

The latter interaction term was no longer significantly related to tHcy in the Industrial Workers population after exclusion of a single outlier with tHcy of 58.5 μmol/L. The interaction between NOS3 genotype and folate was not affected by exclusion of this outlier. There were no obvious outliers in the Young Hearts population. For both populations, the models containing NOS3 terms were significantly better at describing tHcy than models lacking such terms (P<0.0001 for Young Hearts and P=0.0007 for Industrial Workers). This confirms that NOS3 G894T genotype and the interaction between NOS3 genotype and folate are highly significant determinants of tHcy concentration in nonsmokers. The final models explained 41% and 30% of the total amount of variation in tHcy in the Young Hearts and Industrial Workers populations, respectively.

### Discussion

The results of the analyses presented above demonstrate that the NOS3 894TT genotype is a risk factor for elevated tHcy concentrations in healthy nonsmoking adults with low serum folate. This finding held even after adjustment for known determinants of tHcy concentration; namely, folate, vitamin B_{12}, age, sex, and MTHFR C677T genotype. To our knowledge, this is the first report indicating that NOS3 G894T genotype is a determinant of tHcy concentrations.

The tHcy-elevating effect of the NOS3 894TT genotype was only apparent in nonsmokers. This was not surprising, because smoking is known to strongly inhibit NOS3 activity. Thus, in smokers with markedly compromised NOS3 activity, any differential effect on NO and tHcy concentrations attributable to the NOS3 G894T polymorphism would most likely be diminished, and so the risk conferred by the
**NOS3** G894T genotype in the context of smoking would be much smaller.

Median tHcy concentrations associated with the 894TT genotype in nonsmokers were similar to, or higher than, those in smokers irrespective of **NOS3** G894T genotype. In smokers, elevated tHcy concentrations are thought to be one of the smoking-related phenotypic changes that contribute to an increased risk of CVD. It is therefore likely that for nonsmokers with the 894TT genotype, the magnitude of the tHcy-attributable component of CVD risk is similar and may constitute a significant and previously unrecognized risk factor for CVD in a subset of the population that overall has a lower CVD risk than smokers.

Although **NOS3** 894TT homozygotes had the highest tHcy concentrations in nonsmokers from both populations, the impact of the 894GT heterozygote genotype was less consistent. In the Young Hearts population, heterozygotes had tHcy concentrations intermediate between those of 894GG and 894TT homozygotes; however, this was not the case in the Industrial Workers population. More studies will be necessary to determine the impact of the **NOS3** 894GT genotype on tHcy.

The **NOS3** G894T polymorphism has been studied extensively by others to determine whether it results in a functional change in the enzyme. The results of in vivo studies have been inconsistent, and interpretation is difficult because of the different populations, end points, and methodologies used. In vitro data are also inconsistent. Published evidence indicating that in human placental homogenates the Asp298 enzyme encoded by the 894T allele has lower activity than the Glu298 enzyme encoded by the 894G allele is not supported by a subsequent report that in vitro kinetic parameters are similar for the 2 variants of the enzyme.

Although the functional consequences of the **NOS3** G894T polymorphism in a physiological setting have not yet been established, our data can be evaluated in the context of the following 2 plausible mechanisms for NO-mediated modulation of homocysteine concentrations (Figure 1). First, NO inhibits MTR. This hypothesis predicts a positive correlation between NO and homocysteine concentrations, because the substrate homocysteine will accumulate as inhibition of MTR increases. Second, NO inhibits ferritin synthesis. This hypothesis predicts that higher NO concentrations will be associated with decreased ferritin levels and hence higher intracellular folate concentrations. Because folate concentrations are inversely correlated with homocysteine, this would mandate a reciprocal association between NO and homocysteine concentrations.

Our results do not support the hypothesis that NO modifies tHcy concentrations by inhibiting MTR via its vitamin B12 cofactor. Reports to date suggest that the Asp298 form of **NOS3** has either unchanged or reduced activity relative to the Glu298 enzyme. Thus, the MTR-inhibition hypothesis predicts that the 894T allele should be associated with unchanged or lower tHcy concentrations rather than the higher concentrations observed by us. Furthermore, if the effect of **NOS3** 894TT genotype on tHcy concentrations was attributable to differential inhibition of the vitamin B12 cofactor of MTR, tHcy concentrations in subjects with low vitamin B12 should be most susceptible to changes in NO. However, we found no association between **NOS3** G894T genotype and tHcy in subjects from the lowest quartile of vitamin B12 concentrations in either population and no evidence in our models for a significant interaction between **NOS3** genotype and vitamin B12. Finally, we found no evidence for significant interactions between **NOS3** G894T genotype and polymorphisms in MTR or MTRR that affect tHcy concentrations in either population.

The hypothesis that NO reduces intracellular folate catabolism by tonic inhibition of ferritin synthesis, thereby modifying folate-dependent aspects of homocysteine metabolism, is more consistent with our finding that a **NOS3** variant with putative low activity is associated with elevated tHcy and the observation that this relationship is potentiated by low serum folate. Furthermore, the above hypothesis is also supported by our finding of a significant interaction between **NOS3** genotype and serum folate in both populations and a possible interaction between **NOS3** genotype and MTHFR C677T genotype in the Industrial Workers population.

The above relationship is also compatible with the reported link between folate and **NOS3** activity. When the **NOS3** cofactor tetrahydrobiopterin is limiting, **NOS3** has a decreased affinity for arginine together with an increased affinity for O2, which results in the production of superoxide radicals. Evidence suggests that 5-mTHF facilitates tetrahydrobiopterin function and promotes the synthesis of NO rather than superoxide. The Asp298 enzyme may be uniquely sensitive to the effects of suboptimal 5-mTHF. A shift toward increased superoxide production at the expense of NO synthesis could increase folate catabolism and promote a low-folate/high-homocysteine phenotype through the following 2 mechanisms: (1) decreased generation of NO, which would reduce the NO-mediated tonic inhibition of ferritin synthesis; and (2) increased superoxide formation, which would increase the rate of nonenzymatic folate degradation.

In conclusion, we have demonstrated an association between the **NOS3** 894TT genotype and elevated tHcy in nonsmokers with low serum folate from 2 independent populations of healthy adults. This finding is consistent with the hypothesis that NO modulates homocysteine concentrations indirectly by regulating folate catabolism. It is also consistent with the reported negative impact of smoking on total **NOS3** activity. If confirmed by others, studies designed to determine whether **NOS3** G894T genotypes are associated with a range of conditions, including occlusive coronary artery, cerebrovascular, and peripheral vascular diseases, in which a low folate/high homocysteine phenotype has been implicated, are warranted.

**Acknowledgments**

This work was supported by NIH grants AR47633 and HD39195 and in part by grants from Orchid Biosciences, the British Heart Foundation and the Wellcome Trust (Young Hearts population), and Sandoz (Industrial Workers Population). L.A.J. Kluijtmans is supported by grant 1999T023 from the Netherlands Heart Foundation.

**References**


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Arterioscler Thromb Vasc Biol. 2003;23:1014-1020; originally published online April 10, 2003;
doi: 10.1161/01.ATV.0000071348.70527.F4
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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