Effects of NOS3 Glu298Asp Polymorphism on Hemodynamic Reactivity to Stress: Influences of Ethnicity and Obesity

Surender Malhotra, Joseph Poole, Harry Davis, Yanbin Dong, Jennifer Pollock, Harold Snieder, Frank Treiber

Abstract—Studies on the associations between the nitric oxide synthase gene (NOS3) Glu298Asp polymorphism and hypertension status or blood pressure (BP) levels have had inconsistent results. Potential moderating influences of ethnicity, sex, and obesity on the effects of the NOS3 polymorphism have not been examined. We evaluated the influence of these factors on associations between the NOS3 polymorphism, nitric oxide metabolites (NOx), and hemodynamics at rest and during stress. Subjects were 235 African American (AA) and 262 European American (EA) young adults (18.5±2.6 years). Hemodynamic measurements and blood samples for NOx assays were taken before and after a competitive video game challenge. Glu298Asp polymorphism was detected by polymerase chain reaction–restriction enzyme digestion assay. A regression model was built using genotypes, ethnicity, sex, and obesity (body mass index >85th percentile) and their interactions controlling for age; 20.1% of AAs and 49.8% of EAs were carriers of the Asp allele. AAs, regardless of obesity status, exhibited high diastolic blood pressure (DBP) reactivity unless they were nonobese and noncarriers of the Asp allele. EAs exhibited lower DBP reactivity unless they were obese Asp allele carriers. AA nonobese carriers exhibited the greatest total peripheral resistance reactivity. Obese Asp allele carriers exhibited the greatest increases in cardiac output and the greatest decrease in NOx to the stressor. Results indicate the importance of examining impact of BP control-related genetic polymorphisms within the context of moderating factors such as adiposity and ethnicity. (Hypertension. 2004;44:866-871.)

Key Words: blood pressure ■ stress ■ nitric oxide ■ nitric oxide synthase ■ polymorphism

Nitric oxide (NO) is a potent vasodilator that plays a significant role in vasomotor tone and in blood pressure (BP) control. Abrupt interruption of NO synthesis via pharmacological blockade markedly elevates BP in animals and humans.1,2 Individuals with essential hypertension have either diminished whole-body NO production or increased inactivation leading to lower plasma levels.3,4 NO is produced from l-arginine by nitric oxide synthase (NOS), which is a product of the NOS3 gene. The NOS3 gene is located on chromosome 7, spans 21 kb, and contains 26 exons. Several studies have found an Asp for Glu substitution in exon 7 at amino acid residue 298 (Glu298Asp, also called G894T) associated with coronary spasm,5 myocardial infarction,6 coronary artery disease,7 and vascular responsiveness to phentolamine.8 The Asp variant of this polymorphism is believed to render the enzyme more susceptible to proteolytic cleavage.9 There are contradictory results with regard to its association with BP status. Some studies have found Asp allele carrier status to be associated with higher BP levels and increased hypertension prevalence,10 others have observed just the opposite,11 and still others have observed no significant differences based on carrier status.12 This inconsistency across studies has been partly attributed to lack of evaluation of possible modulating influences of subject characteristics known to differentially affect BP such as ethnicity, sex, age, and obesity.11

Exaggerated BP reactivity to stress has been prospectively linked to BP levels and essential hypertension.13 Males, African Americans (AAs), and obese individuals typically show greater BP reactivity as opposed to females, European Americans (EAs), and nonobese individuals, respectively.14–16 However, relatively little attention has been given to the possible association of the Glu298Asp polymorphism with BP reactivity to stress. Rankinen et al17 showed that among participants in an exercise training study, carriers of the Asp allele showed a less beneficial training effect in BP reactivity to a submaximal exercise test compared with noncarriers. The association between Asp carrier status and BP reactivity to behavioral stress has not been examined.

A few studies have examined the relationship between the Glu298Asp polymorphism and plasma nitrite/nitrate (NOx) levels, metabolites of NO, and their findings have been mixed. Asp carrier status has been associated with lower basal NOx levels in one study,18 higher levels in a second...
study,\textsuperscript{19} and a third study found no association.\textsuperscript{20} To our knowledge, associations between the Glu298Asp polymorphism and behavioral stress induced changes in hemodynamic function and NOx levels have not been assessed.

The purpose of the present study was to test the hypothesis that carriers of the Asp allele, particularly those who were males, AAs, or obese would show the highest levels of BP and lowest NOx levels at rest and in response to acute behavioral stress. Because carriers of the Asp allele exhibit their physiological effects through changes in production of a potent vasoactive substance, we hypothesized that the effects of Asp carrier status would be mediated through changes in vascular tone (ie, increased total peripheral resistance [TPR]).

**Methods**

**Study Population**

A total of 497 young adults (235 AAs, 262 EAs; average age, 18.5±2.6 years) participated in the study. Subjects are among the participants in a longitudinal study of the development of biobehavioral risk factors for cardiovascular disease.\textsuperscript{21} All subjects have a verified family history of cardiovascular disease (essential hypertension and/or premature myocardial infarction).\textsuperscript{22} All were normotensive and free of any chronic diseases.

**Protocol**

The study was approved by the institutional review board. After obtaining informed consent, subjects underwent a battery of anthropometric evaluations including height (cm) and weight (kg) using an established protocol.\textsuperscript{21} Hemodynamic measurements were conducted using a bioimpedance monitor (NCCHM-3, Version 6; BoMed Medical Manufacturing Ltd) and Dinamap/Pediatric Vital Signs Monitor (model 1846SX; Critikon). The thoracic bioimpedance monitor measured heart rate and cardiac output. Cardiac output (L/min) was used to calculate TPR as mean arterial pressure/cardiac output (mm Hg/L per min).

After being instrumented for hemodynamic evaluation, the subjects were placed in a supine position. Subjects rested for 20 minutes after a blood draw. Baseline hemodynamics (ie, systolic BP, diastolic BP, and TPR) were calculated based on the average of minutes 13 and 15. After the resting evaluation period, the subjects engaged in a video game challenge (Atari "Breakout," 10 minutes) using a established protocol.\textsuperscript{21} Hemodynamic measurements were conducted by using a bioimpedance monitor (NCCHM-3, Version 6; BoMed Medical Manufacturing Ltd) and Dinamap/Pediatric Vital Signs Monitor (model 1846SX; Critikon). The thoracic bioimpedance monitor measured heart rate and cardiac output. Cardiac output (L/min) was used to calculate TPR as mean arterial pressure/cardiac output (mm Hg/L per min).

After being instrumented for hemodynamic evaluation, the subjects were placed in a supine position. Subjects rested for 20 minutes after a blood draw. Baseline hemodynamics (ie, systolic BP, diastolic BP, and TPR) were calculated based on the average of minutes 13 and 15. After the resting evaluation period, the subjects engaged in a video game challenge (Atari "Breakout," 10 minutes) using a standardized protocol.\textsuperscript{21} Hemodynamics were recorded every 2 minutes. Because the left arm was used for blood drawing, all subjects used their right hand to play the video game. NOx assays were completed as previously described\textsuperscript{23} by using 5-mL blood samples drawn concurrent with the hemodynamic measurements immediately before and after the stressor in a subgroup of subjects (n=205).

**Genotyping**

Genomic DNA was extracted from plasma buffy coats or buccal swabs using the QiaAmp DNA Blood Mini Kit (Qiagen). The Glu298Asp variant of the NOS3 was detected by polymerase chain reaction–restriction enzyme digestion, as described elsewhere.\textsuperscript{24}

**Statistical Analysis**

Results were expressed as mean±SD. Only 27 EAs and none of the AAs were homozygous for the Asp allele. Thus, the genotypes were defined as carriers (GluAsp+AspAsp) and noncarriers (GluGlu) of the Asp allele. We used the change scores as dependent variables and included the baseline values as covariates to account for baseline differences between groups. Analyses of covariance were used to compare the change score values of systolic BP, diastolic BP, TPR, and NOx by ethnicity, sex, carrier status, and standardized body mass index (BMI) after adjusting for age and the baseline values of the dependent variables. We used type III sum of squares, which is a partial least-squares solution that accounts for the variance that is uniquely accounted for by each term in the statistical model after the variance of the other terms in the model have been taken into account. Standardized BMI, based on normative data for age and sex,\textsuperscript{25} was used in the analysis as a continuous variable but was dichotomized for illustrative purposes (Figures 1 to 4) into obese (>85th percentile) and nonobese (<85th percentile).

**Results**

Descriptive characteristics, resting hemodynamics, and NOx levels of the subjects by ethnicity and carrier status are presented in Table 1. AAs were older, heavier, and had higher BMI than EAs (all P<0.001). Noncarriers had higher z scores on standardized BMI compared with carriers (P<0.05). Table 2 shows genotype and allele frequencies by ethnicity. The χ² analysis showed the genotype frequencies to be in Hardy–Weinberg equilibrium in AAs and EAs. The Asp allele frequency was higher in EAs (30% versus 10%; P<0.001).
Hemodynamic Findings: Resting Hemodynamics

Significant differences were noted between AAs and EAs for 4 hemodynamic parameters at rest (all \( P < 0.01 \)). As shown in Table 1, males had significantly higher systolic BP (116.2 versus 108.7 mm Hg; \( P < 0.001 \)), lower diastolic BP (60.8 versus 62.6 mm Hg; \( P < 0.01 \)), and lower TPR (15.8 versus 16.6 mm Hg/L per min; \( P < 0.05 \)) compared with females. AAs exhibited higher resting levels of systolic BP, diastolic BP, and TPR (all \( P < 0.001 \)). There were no other significant main or interaction effects (all \( P > 0.08 \)).

Video Game Reactivity

Reactivity was defined as magnitude of change and calculated by subtracting prestressor levels from the peak response. Reactivity change scores were adjusted for age and baseline values to account for baseline differences. The adjusted change scores were calculated for systolic BP, diastolic BP, TPR, and NOx.

AAs exhibited greater systolic BP (14.3 versus 13.2 mm Hg; \( P < 0.005 \)), diastolic BP (12.0 versus 10.2 mm Hg; \( P < 0.05 \)), and TPR (2.5 versus 2.2 mm Hg/L per min; \( P < 0.05 \)) reactivity compared with EAs. Males also exhibited greater systolic BP (15.8 versus 10.9 mm Hg; \( P < 0.001 \)), diastolic BP (11.5 versus 9.8 mm Hg; \( P < 0.01 \)), and TPR reactivity (2.5 versus 1.9 mm Hg/L per min; \( P < 0.01 \)) compared with females. Carriers of the Asp allele exhibited a greater diastolic BP (11.5 versus 10.2 mm Hg, \( P < 0.05 \)) and TPR reactivity (2.5 versus 2.2 mm Hg/L per min; \( P < 0.01 \)) compared with noncarriers. These main effects for carrier status were qualified by interactions involving ethnicity and obesity, which are shown in Table 3.

A carrier status/ethnicity/obesity status interaction for diastolic BP reactivity (\( P < 0.04 \)) is illustrated in Figure 1. AAs, regardless of obesity status, exhibited greater diastolic BP reactivity compared with EAs, unless they were nonobese and noncarriers of the Asp allele. In contrast, among EAs, diastolic BP reactivity was lower unless they were obese and carriers of the Asp allele. The high diastolic BP reactivity among the AA nonobese carriers was caused by increased vasoconstriction because that group exhibited the greatest TPR reactivity (\( P < 0.03 \)) (Figure 2). An interaction involving obesity and carrier status was seen for CO reactivity (\( P < 0.05 \)), such that obese carriers showed the greatest CO reactivity (Figure 3).

Plasma NOx Findings: Resting Levels

Table 1 shows the resting NOx levels by ethnicity and carrier status. No statistically significant main or interaction effects were observed (all \( P > 0.10 \)).

Video Game Reactivity

Obese individuals exhibited much greater decreases in NOx levels compared with nonobese individuals (−0.93 versus −0.06 \( \mu \)mol/L; \( P < 0.05 \)). An Asp carrier status by obesity interaction (\( P < 0.05 \)) was observed and is depicted in Figure 4. Among noncarriers of the Asp allele, obesity had little impact on adjusted NOx reactivity; however, among carriers, nonobese individuals showed little change whereas obese individuals displayed a marked decrease in adjusted NOx reactivity. There were no other main or interaction effects (all \( P > .10 \)). It should be noted that the Asp carrier status by obesity interaction observed for CO reactivity (\( P < 0.05 \)) was not substantially impacted when the NOx reactivity was used as a covariate (\( P > 0.05 \)).

Discussion

In the present study, we examined whether the Glu298Asp polymorphism in the NOS3 gene was associated with hemodynamic function and NOx levels at rest and in response to an acute behavioral stressor in a sample of EA and AA normo-
Compared with EAs and females, respectively. These ethnic and sex differences are consistent with other pediatric and young adult hemodynamic reactivity studies.\textsuperscript{14,15,22}

Resting hemodynamic function was not statistically different by Asp allele carrier status. Perhaps differences in resting function by carrier status only become apparent after extended years of exposure to environmental factors that potentiate the influence of the polymorphism (eg, nonprudent diet, sedentary behavior, chronic life stress, etc). However, under behavioral stress, Asp allele carrier status did have an impact on hemodynamic function dependent on ethnicity and/or obesity status. That is, Asp allele carrier status appeared to impact BP reactivity via different hemodynamic mechanisms as a function of ethnicity and obesity status. As in this study, BP control tends to be volume-driven in obese\textsuperscript{28} and vasoconstrictive-dominated in AA.\textsuperscript{14,15} Among AAs, nonobese carriers achieved the greatest adjusted diastolic BP reactivity, which was attributed to increased vasoconstriction. Among EAs, obese carriers exhibited the greatest diastolic BP reactivity. With regard to hemodynamic responses to the behavioral stressor that were adjusted for age and baseline values, AAs and males had higher vasoconstrictive-mediated BP reactivity compared with EAs and females, respectively. These ethnic and sex differences are consistent with other pediatric and young adult hemodynamic reactivity studies.\textsuperscript{14,15,22}

Resting hemodynamic function was not statistically different by Asp allele carrier status. Perhaps differences in resting function by carrier status only become apparent after extended years of exposure to environmental factors that potentiate the influence of the polymorphism (eg, nonprudent diet, sedentary behavior, chronic life stress, etc). However, under behavioral stress, Asp allele carrier status did have an impact on hemodynamic function dependent on ethnicity and/or obesity status. That is, Asp allele carrier status appeared to impact BP reactivity via different hemodynamic mechanisms as a function of ethnicity and obesity status. As in this study, BP control tends to be volume-driven in obese\textsuperscript{28} and vasoconstrictive-dominated in AA.\textsuperscript{14,15} Among AAs, nonobese carriers achieved the greatest adjusted diastolic BP reactivity, which was attributed to increased vasoconstriction. Among EAs, obese carriers exhibited the greatest diastolic BP reactivity. With regard to CO reactivity, obese individuals, particularly Asp allele carriers, showed greater adjusted CO reactivity compared with nonobese individuals whose CO reactivity was not affected by carrier status. Relatively few studies have evaluated the impact of obesity on CO reactivity. Jern et al\textsuperscript{28} found that obesity was associated with a CO-dependent vasopressor effect in response to mental stress. Collectively, it appears that Asp allele carrier status tends to exacerbate the vasoconstriction-prone response pattern of nonobese AAs, and the volume-driven BP response pattern of obese.

Previous studies have found mixed results with regard to associations with casual or resting levels of NOx and the NOS3 polymorphism.\textsuperscript{18–20} Similar to our findings, Moon et al\textsuperscript{20} did not find a substantial effect of the NOS3 polymorphism on the plasma NOx levels in a group of 411 healthy Korean subjects aged 19 to 81 years. Interestingly, an ASP carrier status by obesity interaction for NOx reactivity was

Malhotra et al NOS3 Glu298Asp Polymorphism and Obesity

### TABLE 1. Descriptive Statistics by Carrier Status and Ethnicity (Mean±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asp Allele Carrier Status</th>
<th>Ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noncarrier</td>
<td>Carrier</td>
</tr>
<tr>
<td>n</td>
<td>319</td>
<td>178</td>
</tr>
<tr>
<td>Age, y</td>
<td>18.4±2.6</td>
<td>18.4±2.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6±6.7</td>
<td>24.8±7.0</td>
</tr>
<tr>
<td>Standardized BMI, z score</td>
<td>0.7±1.0*</td>
<td>0.4±1.2</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>48</td>
<td>56</td>
</tr>
<tr>
<td>Ethnicity, % AA</td>
<td>59</td>
<td>26</td>
</tr>
<tr>
<td>Obese, %</td>
<td>37</td>
<td>33</td>
</tr>
<tr>
<td>Resting SBP, mm Hg</td>
<td>112.3±10.9</td>
<td>113.1±10.7</td>
</tr>
<tr>
<td>Resting DBP, mm Hg</td>
<td>62.1±7.6</td>
<td>60.9±7.0</td>
</tr>
<tr>
<td>Resting MAP, mm Hg</td>
<td>79.0±7.6</td>
<td>78.3±7.1</td>
</tr>
<tr>
<td>Resting TPR, mm Hg/L/min</td>
<td>16.4±4.6</td>
<td>15.9±4.3</td>
</tr>
<tr>
<td>Resting NOx level, pg/mL</td>
<td>23.7±8.4</td>
<td>23.3±7.8</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
P\textsuperscript{*}P<0.05.
P\textsuperscript{†}P<0.01.
P\textsuperscript{††}P<0.001.

Resting SBP, DBP, MAP, and TPR were statistically different by Asp allele carrier status, and sex differences are consistent with previous findings.\textsuperscript{14,15,22} Males and females had higher vasoconstrictive-mediated BP reactivity compared with EAs and females, respectively. The influence of the polymorphism was exacerbated in obese.

### TABLE 2. Allele and Genotype Frequencies by Ethnicity

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alleles</td>
<td>N</td>
<td>Frequency, %</td>
</tr>
<tr>
<td>Glu</td>
<td>421</td>
<td>90.0</td>
</tr>
<tr>
<td>Asp</td>
<td>47</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>468</td>
<td>526</td>
</tr>
</tbody>
</table>

Genotypes

| Carriers of Asp allele | 47 | 20.1 | 131 | 49.8 |
| Noncarriers of Asp allele | 187 | 79.9 | 132 | 50.2 |
clinical cardiovascular disease, the present findings may have important implications with regard to future cardiovascular disease risk. It can be hypothesized that among AAs, nonobese noncarriers of the ASP allele would be at lowest cardiovascular disease risk. Among EAs, obese Asp allele carriers would be at greatest risk for cardiovascular disease. If future empirical-based evaluations verify such hypotheses, then these classification factors could be used to identify subgroups of individuals at increased risk for stress-induced cardiovascular disease and appropriate prevention programs instituted to deter such risk.

Acknowledgments

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References


<table>
<thead>
<tr>
<th>Variable</th>
<th>P for DBP Reactivity</th>
<th>P for SBP Reactivity</th>
<th>P for CO Reactivity</th>
<th>P for TPR Reactivity</th>
<th>P for NOx Reactivity</th>
</tr>
</thead>
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<tr>
<td>Age</td>
<td>0.00</td>
<td>0.17</td>
<td>0.26</td>
<td>0.00</td>
<td>0.56</td>
</tr>
<tr>
<td>Standardized BMI</td>
<td>0.24</td>
<td>0.49</td>
<td>0.04</td>
<td>0.08</td>
<td>0.64</td>
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<td>Carrier status</td>
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<td>0.45</td>
<td>0.38</td>
<td>0.00</td>
<td>0.06</td>
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<td>Ethnicity</td>
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<td>0.03</td>
<td>0.83</td>
<td>0.02</td>
<td>0.67</td>
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<tr>
<td>Sex</td>
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<td>0.00</td>
<td>0.12</td>
<td>0.01</td>
<td>0.65</td>
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<tr>
<td>Ethnicity by standardized BMI</td>
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<td>0.00</td>
<td>0.65</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Carrier status by standardized BMI</td>
<td>0.76</td>
<td>0.73</td>
<td>0.05</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Sex by standardized BMI</td>
<td>0.10</td>
<td>0.00</td>
<td>0.06</td>
<td>0.77</td>
<td>0.64</td>
</tr>
<tr>
<td>Carrier status by sex</td>
<td>0.78</td>
<td>0.79</td>
<td>0.53</td>
<td>0.98</td>
<td>0.98</td>
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<td>Ethnicity by sex</td>
<td>0.20</td>
<td>0.04</td>
<td>0.07</td>
<td>0.01</td>
<td>0.15</td>
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<tr>
<td>Carrier status by ethnicity</td>
<td>0.14</td>
<td>0.98</td>
<td>0.12</td>
<td>0.09</td>
<td>0.72</td>
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<tr>
<td>Carrier status by ethnicity by BMI</td>
<td>0.04</td>
<td>0.30</td>
<td>0.92</td>
<td>0.03</td>
<td>0.69</td>
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<tr>
<td>Carrier status by ethnicity by sex</td>
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<td>0.53</td>
<td>0.44</td>
<td>0.34</td>
<td>0.28</td>
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<tr>
<td>Carrier status by sex by BMI</td>
<td>0.09</td>
<td>0.53</td>
<td>0.31</td>
<td>0.75</td>
<td>0.29</td>
</tr>
<tr>
<td>Ethnicity by sex by BMI</td>
<td>0.84</td>
<td>0.00</td>
<td>0.64</td>
<td>0.21</td>
<td>0.10</td>
</tr>
<tr>
<td>Carrier status by ethnicity by sex by BMI</td>
<td>0.97</td>
<td>0.56</td>
<td>0.40</td>
<td>0.95</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Reactivity values are covaried for the baseline values.

Observed such that obese carriers of the Asp allele exhibited significantly greater decreases in NOx in response to the stressor compared with the other obesity/allele carrier status subgroups.

Although the findings showed that Asp allele carrier status in the presence of obesity is associated with decreased production of NOx, the possibility exists that this polymorphism is not functional itself, but perhaps in linkage disequilibrium with a functional polymorphism in the regulatory region of the NOS3 gene. That is, the biological significance of the Glu-to-Asp substitution in codon 298 of the NOS3 gene locus is still unclear. The codon is located near the amino-terminal oxygenase domain of the endothelial NOS, which includes binding sites for heme domain, tetrahydrobiopterin, and l-arginine. Whether the Glu298Asp variant has any effect on the binding properties or other functions of the oxygenase domain, or whether it is in linkage disequilibrium with another functional mutation remains to be determined. Finally, because all the subjects had a verified family history of heart disease, whether these findings generalize to other population groups is unknown.

Perspectives

Given that exaggerated hemodynamic reactivity has been shown to play a role in the development of subclinical and clinical cardiovascular disease, the present findings may have important implications with regard to future cardiovascular disease risk. It can be hypothesized that among AAs, nonobese noncarriers of the ASP allele would be at lowest risk for cardiovascular disease. Among EAs, obese Asp allele carriers would be at greatest risk for cardiovascular disease. If future empirical-based evaluations verify such hypotheses, then these classification factors could be used to identify subgroups of individuals at increased risk for stress-induced cardiovascular disease and appropriate prevention programs instituted to deter such risk.

TABLE 3. Main Effects and All the Interactions in the Statistical Model


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