**MAT1A** variants are associated with hypertension, stroke, and markers of DNA damage and are modulated by plasma vitamin B-6 and folate

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**ABSTRACT**

**Background:** The S-adenosylmethionine synthetase type 1 (**MAT1A**) gene encodes a key enzyme in one-carbon nutrient metabolism.

**Objective:** This study aimed to determine the association of **MAT1A** variants with homocysteine, DNA damage, and cardiovascular disease (CVD).

**Design:** Eight variants of **MAT1A** were examined for associations with hypertension, stroke, CVD, homocysteine, and DNA damage in 1006 participants of the Boston Puerto Rican Health Study. Two variants were replicated in 1147 participants of the Nutrition, Aging, and Memory in Elders Study.

**Results:** Two variants and haplotypes were strongly associated with hypertension and stroke, independent of methylenetetrahydrofolate reductase (**MTHFR**) variants. Homozygotes of the **MAT1A** d18777A (rs3851059) allele had a significantly greater likelihood of stroke (odds ratio: 4.30; 95% CI: 1.34, 12.19; \(P = 0.006\)), whereas 3U1510A (rs7087728) homozygotes had a lower likelihood of hypertension (odds ratio: 0.67; 95% CI: 0.48, 0.95; \(P = 0.022\)) and stroke (odds ratio: 0.35; 95% CI: 0.15, 0.82; \(P = 0.015\)). A similar trend of association was observed in a second elderly population. Furthermore, strong interactions between **MAT1A** genotypes and vitamin B-6 status were found. Carriers of the nonrisk allele 3U1510A had a lower 8-hydroxydeoxyguanosine concentration—a biomarker of oxidative DNA damage—when plasma vitamin B-6 was high, whereas homozygotes for the risk-allele 3U1510G had higher 8-hydroxydeoxyguanosine concentrations, regardless of vitamin B-6 status.

**Conclusions:** **MAT1A** variants were strongly associated with hypertension and stroke. Improving folate and vitamin B-6 status might decrease the CVD risk of only a subset of the population, depending on genotype. These findings suggest that impairments in methylation activity, independent of homocysteine, have an effect on CVD risk.


**INTRODUCTION**

Elevated plasma homocysteine (hyperhomocysteinemia) is considered an independent risk factor for cardiovascular disease (CVD). As with traditional risk factors such as dyslipidemia (1, 2), the risk associated with hyperhomocysteinemia is hypothesized to be modifiable. However, the failure of a series of recent clinical trials (3–6) to reduce recurrent CVD risk by lowering homocysteine with B vitamin therapy has called this hypothesis into question and has left the significance of the strong epide-
good in vivo data in animal models (15, 16) and in humans (17–19) suggest that changes in methylation, SAM, or methio-
nine metabolism may be associated with CVD independently of homocysteine (16–20). Hence, genetic, nutritional, or physi-
ologic impairments to methylation activity could simultaneously
cause imbalances in SAM, SAH, homocysteine, and methionine,
each of which could affect vascular risk. In this context we
sought to test the hypothesis that genetic variation in the MAT1A
gene would modify age-related CVD risk in humans. Methio-
nine adenosyltransferase genes have been identified as a phy-
logenetically conserved determinant of longevity in model
organisms (21, 22). MAT1A knockout mice exhibit severe SAM
deficiency and hypermethioninemia, but show no alteration in
homocysteine concentrations (23), whereas human patients with
certain MAT1A mutations exhibit hypermethioninemia (24) and
neurologic deficits (25). We were therefore interested in
determining the importance of genetic variation of MAT1A on
the risk of CVD in humans.

The population of Puerto Rican adults living in Massachusetts
has a disproportionate health burden, including a high prevalence
of hypertension, diabetes, obesity, and CVD (26, 27). In this
study, we examined the association of genetic variation in
MAT1A with stroke, hypertension, and related traits in the
Boston Puerto Rican Health Study (BPRHS) (27). We then
replicated associations in another Boston-based study of a co-
hort at high risk of CVD—the Nutrition, Aging, and Memory in
Elders (NAME) Study (28).

SUBJECTS AND METHODS

Study population and methods

The BPRHS and NAME Study were conducted by a core team
of investigators at Tufts University, who used similar methods
and a shared core laboratory. Written informed consent was
obtained from every participant, and both protocols were ap-
proved by the Institutional Review Board at Tufts University.

The BPRHS Study

This study sample consisted of 1006 self-identified Puerto
Ricans (299 men and 707 women) living in the greater Boston
metropolitan area and for whom full data records for demogra-
phic characteristics, biochemical measures, and genotypes were
available. These participants were recruited by investigators from
the Boston Puerto Rican Center for Population Health and Health
Disparities to participate in a longitudinal cohort study on stress,
nutrition, health, and aging—the BPRHS (27; http://cphhd.hnrc.
tufts.edu/es?theme=bprhs). A detailed description of the popula-
tion of Puerto Rican adults living in Massachusetts
was obtained from every participant, and both protocols were ap-
proved by the Institutional Review Board at Tufts University.

The NAME Study

The NAME Study was designed to examine associations
between micronutrient deficiencies and cognitive impairment in
community-living high-risk adults aged ≥60 y receiving home-
care services (28). Participants were recruited through area
Aging Services Access Points, which are home-care agencies
that provide the services and support needed to make in-
dependent living possible for the elderly in the greater Boston
area (28). This study included 1147 predominantly self-reported
European and African American participants (275 men and 872
women), for whom phenotype and genotype data pertinent to
this study were available and who enrolled in the NAME Study

Data collection and variable definition

Anthropometric measurements were collected by using stan-
dard methods. Hypertension was identified on the basis of high
systolic (>140 mm Hg) or diastolic (>90 mm Hg) blood pres-
sure, from the average of the latter 2 of 3 blood pressure
measurements or reported use of blood pressure medication.
The latter 2 measures are used to reduce the possibility of artificially
high measures due to anxiety during the first measure. History of
stroke and of CVD was self-reported from the following ques-
tions: “Has a doctor ever told you that you had a stroke?” and
“What have you ever been told by a physician that you had heart
disease?” Participants were classified as having type 2 diabetes
on the basis of American Diabetes Association criteria (29) if
a fasting plasma glucose concentration ≥126 mg/dL or use of
insulin or diabetes medication was reported. Physical activity
was estimated as a physical activity score based on the Paffen-
barger questionnaire of the Harvard Alumni Activity Survey
(30). Fasting blood samples were drawn by a certified phlebot-
omist. Aliquots were saved and stored at −80˚C until processed.

Plasma assessment

Total plasma homocysteine was measured by using HPLC with
fluorescence detection (31). Plasma vitamin B-6 (or pyridoxal
phosphate) was measured by using the radioenzymatic method of
Camp et al (32). Plasma folate and vitamin B-12 were measured
by using Immulite Chemiluminescent kits according to the
manufacturer’s instructions (Diagnostic Products Corporation/
Siemens, Los Angeles, CA). Plasma creatinine was measured by
using a modification of the Jaffe method (33).

DNA isolation and genotyping

Genomic DNA was isolated from buffy coats of peripheral
blood by using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden,
Germany) according to the vendor’s recommended protocol.
Single nucleotide polymorphisms (SNPs) were genotyped with
Applied Biosystems TaqMan SNP genotyping system (34).

Urinary 8-OHdG and DNA damage

Oxidative DNA damage and the whole-body repair of DNA
were estimated (26, 35) in the BPRHS only by measuring
8-hydroxydeoxyguanosine (8-OHdG) in urine samples with
a monoclonal antibody ELISA kit (catalog no. EKS-350) from
Assay Designs (Ann Arbor, MI). Concentrations of urinary
8-OHdG were normalized against the total amount of creatinine
in the urine (ng/mg creatinine).

MAT1A SNP selection

A panel of 8 SNPs mapping in/near the MAT1A gene (Table 1)
were selected for genotyping, based primarily on a linkage
disequilibrium (LD) analysis of HapMap data for the CEU
population (www.hapmap.org). Results of Tagger (36), run
with the parameters of pairwise option, CEU population, r² >
0.80, minor allele frequency (MAF) >0.05, placed most SNPs
into 1 of 11 blocks. Each of the SNPs chosen for genotyping
TABLE 1

Characteristics of 8 MAT1A single nucleotide polymorphisms (SNPs) genotyped in the Boston Puerto Rican Health Study (BPRHS) and the Nutrition, Aging, and Memory in Elders (NAME) Study populations.

<table>
<thead>
<tr>
<th>SNP name</th>
<th>NCBI accession (rs no.)</th>
<th>HGVS name</th>
<th>Distance from TSS (bp)</th>
<th>Gene region</th>
<th>BPRHS: minor allele frequency (SE)</th>
<th>NAME: minor allele frequency (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT1A_m1038</td>
<td>rs7919385</td>
<td>NT_030059.12g.798988A→G</td>
<td>-1038</td>
<td>Upstream</td>
<td>0.01 (0.01)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_i1262</td>
<td>rs9421467</td>
<td>NM_000429.2c.346+916G→C</td>
<td>1262</td>
<td>Intron 1</td>
<td>0.07 (0.01)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_A1422</td>
<td>rs1143694</td>
<td>NM_000429.2c.681G→A</td>
<td>9383</td>
<td>Exon 5</td>
<td>0.19 (0.01)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_i15713</td>
<td>rs2994388</td>
<td>NM_000429.2c.1340+14A→G</td>
<td>15,173</td>
<td>Intron 8</td>
<td>0.40 (0.02)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_3U1510</td>
<td>rs7087728</td>
<td>NM_000429.2c.1510G→A</td>
<td>15,752</td>
<td>Intron 8</td>
<td>0.20 (0.01)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_i15722</td>
<td>rs4933327</td>
<td>NM_000429.2c.1341→11G→A</td>
<td>15,965</td>
<td>3′-UTR</td>
<td>0.25 (0.01)</td>
<td>0.22 (0.01)</td>
</tr>
<tr>
<td>MAT1A_d18777</td>
<td>rs3851059</td>
<td>NT_030059.12g.797174G→A</td>
<td>18,777</td>
<td>Downstream</td>
<td>0.30 (0.02)</td>
<td>0.26 (0.01)</td>
</tr>
<tr>
<td>MAT1A_d28866</td>
<td>rs10887701</td>
<td>NT_030059.12g.769085C→T</td>
<td>28,866</td>
<td>Downstream</td>
<td>0.41 (0.02)</td>
<td>—</td>
</tr>
</tbody>
</table>

1 bp, base pairs; TSS, transcription start site (distance in bp along the chromosome); NCBI, National Center for Biotechnology Information; UTR, untranslated region.

2 SNP name: m indicates that the SNP is located in the promoter or upstream of the gene, i is in the intron, and d is downstream or beyond the transcribed region.

3 HGVS names of SNPs are based on nomenclature recommendations of the Human Genome Variation Society (http://www.hgvs.org/rec.html).

Furthermore, the table indicates that the variation in MAT1A is associated with hypertension and stroke. The study suggests that differences in population admixture are important in understanding these associations. The population admixture is a measure of the genetic diversity within a population, and it is influenced by historical events such as migration and selection. The admixture analysis in the study suggests that the differences in the admixture proportions between the BPRHS and NAME populations may explain the observed differences in the prevalence of hypertension and stroke. The study also highlights the importance of understanding the genetic variation within populations to inform public health strategies.
TABLE 2
Characteristics of the Boston Puerto Rican Health Study (BPRHS) and the Nutrition, Aging, and Memory in Elders (NAME) Study populations according to sex

<table>
<thead>
<tr>
<th></th>
<th>BPRHS</th>
<th>NAME</th>
<th>P for BPRHS vs NAME (t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n = 299)</td>
<td>Women (n = 707)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>57.4 ± 7.8d</td>
<td>57.6 ± 7.3</td>
<td>—</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>137.7 ± 18.4</td>
<td>155.3 ± 18.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>82.7 ± 11.2</td>
<td>80.5 ± 10.3</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.6 ± 5.1</td>
<td>33.0 ± 6.99</td>
<td>0.037</td>
</tr>
<tr>
<td>Physical activity score</td>
<td>32.5 ± 5.7</td>
<td>31.1 ± 4.0</td>
<td>0.114</td>
</tr>
<tr>
<td>Plasma folate (ng/mL)</td>
<td>17.8 ± 8.6</td>
<td>20.1 ± 9.34</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma vitamin B-12 (pg/mL)</td>
<td>529.9 ± 271.3</td>
<td>546.8 ± 278.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Plasma creatinine (mg/L)</td>
<td>124.2 ± 69.2</td>
<td>143.1 ± 75.1</td>
<td>0.006</td>
</tr>
<tr>
<td>Plasma homocysteine (µmol/L)</td>
<td>10.7 ± 5.9</td>
<td>8.7 ± 4.1d</td>
<td>0.001</td>
</tr>
<tr>
<td>Cardiovascular diseases [n (%)]</td>
<td>72 (24.1)</td>
<td>142 (20.1)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

1 NA, not applicable; 8-OHdG, 8-hydroxydeoxyguanosine.
2 Calculated by using a t test for mean differences between 2 populations.
3 Mean ± SD (all such values).
4 Statistical significance at P < 0.05 within population.

hypothesis was 15% more prevalent, CVD was twice as prevalent, and stroke was 5 times as prevalent in the NAME Study as in the BPRHS (Table 2). Compared with the BPRHS population, men and women of the NAME Study had similar patterns of differences in BMI, homocysteine, creatinine, and smoking, with the exception of plasma folate, vitamin B-12, alcohol drinking, and type 2 diabetes. Plasma folate was significantly lower in the NAME Study than in the BPRHS in men (P = 0.011) and women (P < 0.001). Plasma vitamin B-12 and vitamin B-6 were significantly higher in the NAME Study women than in the BPRHS women (P = 0.005 and 0.006, respectively), whereas no significant difference in men were observed between the 2 populations. In addition, type 2 diabetes was significantly less prevalent in women than in men in the NAME Study.

In the BPRHS, an MAF of 8 MAT1A SNPs ranged from 0.01 to 0.41 (Table 1). Except for SNP 3U1510 (P = 0.031), all SNPs were in Hardy-Weinberg equilibrium. Pairwise LD among the 8 SNPs varied (data not shown) from little LD (r² = 0.03) to moderate LD (r² = 0.75). SNPs 3U1510 and d18777 were in weak LD (r² = 0.39). In the NAME Study, SNPs 3U1510 and d18777, with MAFs of 0.22 and 0.26, respectively, were both in Hardy-Weinberg equilibrium and in weak LD (r² = 0.27).

Association of MAT1A variants and hypertension, stroke, and CVD

Because no genotype-by-sex interaction was observed, all analyses were conducted by combining data for men and women. In the BPRHS, SNP 3U1510 (rs7087728) was significantly associated with stroke [odds ratio (OR): 0.35; 95% CI: 0.15, 0.82; P = 0.015] and with hypertension (OR: 0.67; 95% CI: 0.48, 0.95; P = 0.022) for AA+AG vs GG, as evident in Table 3. GG carriers had a nearly 3-fold higher likelihood of stroke than did AA carriers. SNP d18777 (rs3851059) was also significantly associated with stroke (OR: 4.04; 95% CI: 1.34, 12.19; P = 0.022), but not with hypertension (P = 0.154). In this case, homozygotes (AA) of the minor allele had a 4 times greater likelihood of stroke than did homozygotes of the major allele (GG), whereas heterozygotes (AG) did not differ significantly from homozygotes (GG) (OR: 0.87; 95% CI: 0.36, 2.11). The OR for stroke in the d18777 homozygotes (AA) compared with the combined group of heterozygotes (AG) and homozygotes (GG) was 4.30 (95% CI: 1.52, 12.15; P = 0.006). In contrast, only a marginal association with CVD was noted for SNPs 3U1510 (OR: 0.72; 95% CI: 0.50, 1.04; P = 0.077) and d18777 (OR: 2.02; 95% CI: 1.13, 3.59; P = 0.017) for AA vs AG+GG. The other 6 variants showed no significant association with either disease. No variants had a significant association with blood pressure (data not shown).

To confirm associations between MAT1A variants 3U1510 and d18777 with stroke and hypertension, we genotyped both SNPs in the NAME Study population. SNP 3U1510 had a similar, but nonsignificant, direction of association with stroke (OR: 0.72; 95% CI: 0.45, 1.14; P = 0.161; n = 635) for AA+AG vs GG in European Americans. Similarly, d18777 was marginally associated with stroke (OR: 2.83; 95% CI: 0.87, 9.21; P = 0.084; n = 349) for AA vs AG+GG in African Americans. However, associations between these 2 SNPs and hypertension were not significant in either group (data not shown).

Interaction of MAT1A genotypes with folate and vitamin B-6 on homocysteine

To determine whether the association between MAT1A variants, hypertension, and stroke might be explained through an
effect on plasma homocysteine, we examined associations between MAT1A genotypes and plasma homocysteine concentrations in the BPRHS. We found no significant association between 3U1510 genotypes and homocysteine concentrations (AG+GG compared with GG: 10.2 ± 0.3 compared with 10.0 ± 0.3 μmol/L; P = 0.348). However, homozygous individuals (AA) for the d18777 SNP, who had a greater likelihood of stroke, had significantly higher homocysteine concentrations than did individuals carrying either of the other 2 genotypes (AG+GG vs AA: 10.6 ± 0.5 compared with 9.7 ± 0.3 μmol/L; P = 0.019). Finally, SNP i15173 had an association with homocysteine that just failed to meet the significance criterion (P = 0.056), whereas the other 5 variants were not associated with plasma homocysteine concentrations (data not shown).

In the BPRHS, we then tested for possible gene-nutrient interactions by analyzing the association of MAT1A genotypes with homocysteine as a function of folate, vitamin B-6, or vitamin B-12 status. We divided the cohort into 2 groups, based on the mean of each plasma vitamin measure, after the exclusion of individuals with extreme values (folate > 70 ng/mL, vitamin B-12 > 2000 pg/mL, vitamin B-6 > 300 nmol/L). We observed that both SNPs 3U1510 and d18777 showed significant interactions with plasma folate by influencing plasma homocysteine concentrations (P = 0.039 and 0.032, respectively; Figure 1). As expected, individuals who had lower plasma folate concentrations (<19 ng/mL) had higher homocysteine concentrations (10.5–12.2 μmol/L), whereas those with higher plasma folate concentrations (>19 ng/mL) had lower plasma homocysteine concentrations (8.0–9.0 μmol/L). When stratified by folate status, homozygous individuals with the risk allele d18777A and low folate status (<19 ng/mL) had significantly higher homocysteine concentrations (12.2 compared with 10.5 μmol/L; P = 0.004) than did the rest of the population (AG+GG). However, those with a higher folate status (>19 ng/mL) showed no significant differences across d18777 genotypes (8.0 compared with 8.3 μmol/L; P = 0.939). In contrast, SNP 3U1510 showed no significant difference in homocysteine by genotype in those with low folate status (<19 ng/mL): 10.9 compared with 11.1 μmol/L (P = 0.682). However, homozygotes of the 3U1510 G allele had lower homocysteine concentrations in those with high folate status (8.3 compared with 9.0 μmol/L; P = 0.027). Neither vitamin B-6 nor vitamin B-12 influenced plasma homocysteine differently by genotype (data not shown).

### Table 3

Association between MAT1A variants and hypertension and stroke in the Boston Puerto Rican Health Study

<table>
<thead>
<tr>
<th>SNP name</th>
<th>Genotype</th>
<th>n</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT1A_m1038</td>
<td>AG+GG vs AA</td>
<td>27 vs 971</td>
<td>1.57</td>
<td>0.19, 12.73</td>
<td>0.675</td>
<td>0.70</td>
<td>0.26, 1.92</td>
<td>0.490</td>
<td>1.41</td>
<td>0.50, 4.00</td>
<td>0.519</td>
</tr>
<tr>
<td>MAT1A_i1262</td>
<td>CC+CG vs GG</td>
<td>133 vs 834</td>
<td>1.23</td>
<td>0.43, 3.50</td>
<td>0.699</td>
<td>0.80</td>
<td>0.50, 1.27</td>
<td>0.339</td>
<td>1.10</td>
<td>0.65, 1.73</td>
<td>0.731</td>
</tr>
<tr>
<td>MAT1A_A142A</td>
<td>AA+AG vs GG</td>
<td>340 vs 630</td>
<td>0.95</td>
<td>0.44, 2.06</td>
<td>0.898</td>
<td>1.13</td>
<td>0.80, 1.60</td>
<td>0.351</td>
<td>0.93</td>
<td>0.64, 1.34</td>
<td>0.679</td>
</tr>
<tr>
<td>MAT1A_i15173</td>
<td>AA vs GG</td>
<td>349 vs 163</td>
<td>0.36</td>
<td>0.13, 1.01</td>
<td>0.152</td>
<td>0.60</td>
<td>0.36, 1.00</td>
<td>0.145</td>
<td>0.78</td>
<td>0.47, 1.29</td>
<td>0.610</td>
</tr>
<tr>
<td>MAT1A_i15752</td>
<td>AG vs GG</td>
<td>441 vs 163</td>
<td>0.62</td>
<td>0.25, 1.54</td>
<td>0.493</td>
<td>0.69</td>
<td>0.42, 1.12</td>
<td>0.384</td>
<td>0.87</td>
<td>0.54, 1.41</td>
<td>0.484</td>
</tr>
<tr>
<td>MAT1A_3U1510</td>
<td>AG+AA vs GG</td>
<td>429 vs 497</td>
<td>0.35</td>
<td>0.15, 0.82</td>
<td>0.015</td>
<td>0.67</td>
<td>0.48, 0.95</td>
<td>0.022</td>
<td>0.72</td>
<td>0.50, 1.04</td>
<td>0.077</td>
</tr>
<tr>
<td>MAT1A_d18777</td>
<td>AA vs GG</td>
<td>82 vs 455</td>
<td>4.04</td>
<td>1.34, 12.19</td>
<td>0.022</td>
<td>0.75</td>
<td>0.42, 1.32</td>
<td>0.154</td>
<td>2.09</td>
<td>1.14, 3.82</td>
<td>0.055</td>
</tr>
<tr>
<td>MAT1A_d28866</td>
<td>CC vs TT</td>
<td>337 vs 166</td>
<td>0.43</td>
<td>0.16, 1.16</td>
<td>0.238</td>
<td>0.73</td>
<td>0.45, 1.19</td>
<td>0.204</td>
<td>0.60</td>
<td>0.35, 0.99</td>
<td>0.097</td>
</tr>
</tbody>
</table>

*P* values were calculated by logistic regression models and adjusted for MTHFR genotype, age, sex, BMI, smoking, alcohol use, physical activity, medication use for depression and type 2 diabetes, population admixture, and plasma concentrations of vitamin B-6, vitamin B-12, folate, and creatinine.

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**Figure 1.** Interaction effects of MAT1A single nucleotide polymorphism 3U1510 (A) and single nucleotide polymorphism d18777 (B) with plasma folate concentrations on homocysteine concentrations in individuals with either the AA or AG genotype (AA+AG, nonrisk-allele carriers; unshaded bars) or the GG genotype (risk-allele homozygote; shaded bars) (panel A) and in individuals with either the AG or GG genotype (AG+GG, nonrisk-allele carriers; unshaded bars) or the AA genotype (risk-allele homozygote; shaded bars) (panel B). The numbers inside the bars indicate the number of participants with a given genotype. *P* values for interactions between genotype and folate concentrations and for difference between genotypes are provided above the bars.
Interaction of \textit{MAT1A} genotypes with folate and vitamin B-6 on DNA damage

Good evidence indicates that homocysteine-induced oxidative stress and impairments in methylation activity due to vitamin deficiency or genetic defects could lead to DNA damage and CVD risk (11–14, 42). Urinary 8-OHdG is a widely used marker of in vivo oxidative DNA damage (43), and, in animal models, 8-OHdG can be experimentally modified by folate and vitamin B-6 (44). Therefore, we assessed whether \textit{MAT1A} variants were associated with the oxidative DNA damage marker 8-OHdG and whether it was modulated by plasma vitamin B-6 status.

There was no main effect of \textit{MAT1A} genotype on DNA damage. However, after stratification by vitamin B-6 status, we observed a strong interaction between 3U1510 genotype and vitamin B-6 status on DNA damage ($P < 0.001$). Homozygotes of the risk allele with lower plasma vitamin B-6 concentrations ($<55$ nmol/L) had significantly less DNA damage than did the other 2 genotypes (GG: 128 ng/mg; AA+AG: 144 ng/mg; $P = 0.026$; Figure 2A). However, such homozygotes with higher vitamin B-6 concentrations ($>55$ nmol/mL) had significantly more DNA damage than did the other 2 genotypes combined (GG: 135 ng/mg; AA+AG: 117 ng/mg; $P = 0.011$) after adjustment for potential confounders (see Statistical analysis).

To determine whether vitamin B-6 status modulated the association between 3U1510 genotype and DNA damage in a dose-dependent manner, we treated vitamin B-6 as a continuous variable. Consistent with the result for the categorical variable, we found a significant interaction between plasma vitamin B-6 concentration and 3U1510 genotype on DNA damage (Figure 2B; $P = 0.009$). As plasma vitamin B-6 increased, the amount of DNA damage decreased in carriers of the nonrisk allele (AA+AG). In contrast, DNA damage remained high in individuals homozygous for the risk allele (GG) across vitamin B-6 concentrations (Figure 2B). This indicates that individuals homozygous for the risk allele (GG), who represented 54% of the population, may less efficiently use vitamin B-6-dependent pathways in resisting oxidative DNA damage than noncarriers. Despite interactions between SNP 3U1510, folate, and homocysteine, plasma folate did not modulate the association between genotype and DNA damage (data not shown). Furthermore, B vitamins showed no significant interactions with SNP d18777 or any other \textit{MAT1A} SNP on 8-OHdG concentrations in this population (data not shown).

Association between \textit{MAT1A} haplotypes, stroke, hypertension, CVD, and DNA damage

To examine the combined effect of \textit{MAT1A} genetic variants on stroke, hypertension, CVD, and DNA damage in the BPRHS, we performed haplotype analysis based on 3 \textit{MAT1A} variants (i15173, 3U1510, and d18777), all of which showed significant associations ($P < 0.050$) or an association with a $P$ value $<0.200$ with the phenotypes of interest (ie, stroke, hypertension, and DNA damage). For these 3 SNPs, we observed 5 haplotypes: G-G-G, A-G-A, A-A-G, A-G-G, G-G-A, with frequencies ranging from 0.03 to 0.37, accounting for 100% of all haplotypes in the population (Table 4). After correction for multiple tests, \textit{MAT1A} haplotypes showed a significant global association with stroke ($P = 0.002$). In particular, haplotype A-A-G (frequency = 0.26) was associated with a significantly lower likelihood of stroke (OR: 0.27; 95% CI: 0.11, 0.65; $P = 0.003$) compared with noncarriers. \textit{MAT1A} haplotypes also correlated significantly with hypertension ($P = 0.020$) after permutation test correction. Consistently, carriers of the haplotype had a lower likelihood of stroke (A-A-G) and a significantly lower likelihood of hypertension (OR: 0.63; 95% CI: 0.46, 0.87; $P = 0.005$) than did noncarriers. However, the association between \textit{MAT1A} haplotypes and CVD did not reach global significance (data not shown). Interestingly, when heart attack was excluded from CVD, \textit{MAT1A} haplotypes showed a globally significant association with CVD ($P = 0.025$), such that the risk associated with each haplotype for CVD was similar to that of stroke (Table 4).

Further examinations of correlations between \textit{MAT1A} haplotypes and homocysteine and DNA damage showed that \textit{MAT1A} haplotypes were not directly associated with 8-OHdG ($P = 0.917$) or with plasma homocysteine concentrations ($P = 0.486$). However, a strong interaction ($P = 0.0004$) between haplotype A-A-G and plasma vitamin B-6 on DNA damage was observed (Table 5). Individuals with the A-A-G haplotype tended to have greater DNA damage (146 compared with 130 ng/mg creatinine; $P = 0.036$) than did those without this haplotype when plasma vitamin B-6 was <55 nmol/L. In contrast, these individuals had significantly less DNA damage (113 compared with 129 ng/mg creatinine; $P = 0.020$) than did those without this haplotype, when plasma vitamin B-6 was ≥55 nmol/L. We observed no significant interaction between \textit{MAT1A} haplotypes and folate status on DNA damage and no interaction with...
plasma vitamin B-6, vitamin B-12, or folate status on plasma homocysteine concentrations (data not shown).

**DISCUSSION**

*MAT1A* encodes hepatic methionine adenosyltransferase I/III, the enzyme responsible for the synthesis of SAM. Despite the existence of an alternate *MAT2A* gene in extrahepatic tissue, MAT I/III is quantitatively necessary to maintain adequate whole-body methylation capacity and sulfur amino acid metabolism (20). In theory, functional variation in *MAT1A* gene products and expression could alter flux through pathways that require its product SAM for methylation activity or polyamine synthesis, for maintaining the normal flux of sulfur amino acids through the methionine and transsulfuration pathways, or for the coordinated regulation of these pathways with folate metabolism through regulation by SAM of enzymes such as MTHFR (45).

Here, we show that *MAT1A* variants and haplotypes are significantly associated with hypertension and stroke after adjustment for *MTHFR* genotype as well as other potential confounding factors (ie, age, sex, BMI, physical activity score, population admixture, medication use for diabetes and depression, and plasma concentrations of folate, vitamins B-6, vitamin B-12, and creatinine). Thus, the association of *MAT1A* variation with stroke and hypertension is independent of *MTHFR*.

Our results indicate that the direction of the interaction between *MAT1A* genotype and vitamin B-6 in relation to DNA damage was consistent with the observation for the relation with stroke and hypertension. With greater plasma vitamin B-6, carriers of the risk allele had more DNA damage than did noncarriers. This implies that, as plasma vitamin B-6 increases, carriers of the nonrisk allele may be protected against stroke and hypertension because of lower DNA damage, whereas the risk-allele carriers are not protected. Furthermore, our observation that *MAT1A* genotype showed a strong interaction with vitamin B-6 on DNA damage suggests that *MAT1A* genotype may influence the potential for cardiovascular protection through an improvement in vitamin B-6 status. Such potential confounding by *MAT1A* genotypes (and similar gene-nutrient interactions) might have contributed to the failure of homocysteine-lowering trials, most of which used high-dose vitamin B-6 to lower CVD risk (3–6). For example, the Norwegian Vitamin Trial (6)—which compared cardiovascular outcomes in subjects randomly assigned to receive placebo, 40 mg vitamin B-6 alone, 0.8 mg folate + 400 μg vitamin B-12 alone, or all 3 vitamins—found no difference in the probability of an event between the placebo, folate + vitamin B-12, and the vitamin B-6 only group. However, the group that received all 3 vitamins had a 22% higher risk than did the control subjects (relative risk: 1.22; 95% CI: 1.0, 1.5; *P* = 0.05). The result was significant, but marginally so,
and raised questions as to whether vitamin B-6 might be harmful through some unrecognized interaction with folate and vitamin B-12–dependent metabolism. It is possible that such interactions might be influenced by \textit{MAT1A} or other genotypes. In addition, a hypothesis-driven post hoc subgroup analysis of trials showed significant benefits of supplementation in those groups most likely to benefit, even when the primary intention-to-treat analysis across the entire trial population was null. For example, despite null findings in the Vitamin for Stroke Prevention Trial (VISPT) (46), analysis by baseline B-12 status showed significantly improved survival as a function of baseline vitamin B-12 status and supplementation (47). Our findings suggest that a similar benefit may accrue to significant segments of the population as a function of genotype (Figure 2B). This hypothesis could be tested in future studies and in focused post hoc analyses of completed trials such as NORVIT.

Elevated plasma homocysteine has been identified as a risk factor for CVD. We found that the d18777A risk allele was associated with higher homocysteine concentrations generally, and both variants 3U1510 and d18777 interacted with folate status, albeit by influencing plasma homocysteine in opposite directions (Figure 1). However, none of the haplotypes—based on an analysis with 3 \textit{MAT1A} SNPs (i15173, 3U1510, and d18777)—showed a significant interaction with plasma folate in relation to homocysteine. On the other hand, our results showed that individuals with higher folate had lower homocysteine, regardless of their genotype. This suggests that improving folate status lowers homocysteine regardless of \textit{MAT1A} genotype, which is consistent with previous observations (1).

Recent clinical studies with supplemental B vitamins and folate to reduce homocysteine showed little benefit on CVD prevention, especially secondary prevention (3–6). Thus, elevated plasma homocysteine may not be a causal factor of CVD. In this study, we found that the association between \textit{MAT1A} variants and stroke and hypertension remained significant even after adjustment for homocysteine (data not shown). In addition, we further examined the correlation between DNA damage and CVD (combined stroke, heart disease, and heart attack). Whereas there was no direct significant association between DNA damage and CVD, we did see a significant or trend of association between DNA damage and CVD in carriers of the risk allele or genotype (d18777 and 3U1510). Among carriers of d18777 AA genotypes, subjects with CVD tend to have greater DNA damage (151.3 ± 9.2 compared with 132.9 ± 3.3 ng/mg, \(P = 0.005\)) than unaffected subjects. Similarly, among carriers of 3U1510 GG genotype, subjects with CVD had slightly greater DNA damage (140.9 ± 6.6 compared with 133.2 ± 3.5 ng/mg; \(P = 0.199\)) than did subjects without CVD. However, among noncarriers, there was no significant difference (\(P = 0.599\) and 0.453, respectively) in DNA damage. These observations in combination with the strong interaction between vitamin B-6 and \textit{MAT1A} genotype on DNA damage suggest an alternate hypothesis: CVD-associated \textit{MAT1A} variants contribute to CVD risk, not through homocysteine but, rather, through mechanisms that are involved in impairments in methylation activity and oxidative stress, which remains to be elucidated.

We selected 8 independent SNPs (ie, no strong LD among them) to maximize the coverage of the \textit{MAT1A} region, but only 2 variants showed a significant association with stroke and hypertension. Although none of the associations individually passed Bonferroni correction (\(P = 0.003\)), the \textit{MAT1A} haplotypes consisting of 3 SNPs did show a significant association with hypertension, stroke, and CVD even after permutation test correction. Whereas there was a limited number of individuals with stroke (\(n = 39\)) in this population, the number with hypertension was substantial (\(n = 700\)). In addition, both SNPs (3U15010 and d18777) that were associated with stroke were also either significantly associated with hypertension or nearly significantly associated (\(P = 0.022\) and \(P = 0.154\), respectively). These 2 SNPs also showed similar trends of association with stroke in a second elderly population, although these SNPs may not be the best proxies of \textit{MAT1A} variants that are associated with CVD risk in the NAME Study population. Additionally, haplotypes consisting of both SNPs showed consistent associations with stroke, hypertension, and CVD (Table 4). Considering the fundamental importance of \textit{MAT1A} function in homocysteine metabolism and other critical biological processes, and the strong interaction (\(P = 0.0004\)) of \textit{MAT1A} haplotype with plasma vitamin B-6, we believe that our identification of 2 specific \textit{MAT1A} variants that were associated with stroke and hypertension was not by chance.

Our discovery of \textit{MAT1A} variants as candidate risk factors for CVD in humans is consistent with a broader role of SAM-dependent metabolism in aging. We initially identified the homolog of mammalian \textit{MAT1A}, \textit{M(2)21AB} (CG2674), as a longevity gene in \textit{Drosophila}, where aged male flies had 40% higher expression of \textit{M(2)21AB} than did young male flies (22). Consistent with this observation, \textit{MAT1A} is more highly expressed in the heart ventricle of old rats than in young rats (48). In addition, exercise reduced \textit{MAT1A} expression in the heart ventricle of rats (49), whereas rats subjected to acute hypotension had increased expression in the kidney (50). In 2 independent studies of the adult nematode \textit{Caenorhabditis elegans}, RNAi knockdown expression of the \textit{MAT1A/MAT2A} homolog, \textit{Y105C5B.12}, led to extension of the life span by 15–27% (21). These observations support the hypothesis that a reduction in \textit{MAT1A} expression in postdevelopment (adults) might curtail the process of aging or aging-related phenotypes. Our observation that \textit{MAT1A} variants are associated with hypertension, stroke, homocysteine, and DNA damage could be consistent with a role for \textit{MAT1A} in the aging process, although the relation between \textit{MAT1A} genotype and SAM-dependent metabolism in vascular and other age-related diseases remains to be determined.

In summary, we showed that genetic variation at the \textit{MAT1A} locus, which encodes a key enzyme at the nexus of methylation, polyamine, folate, and sulfur amino acid metabolism and whose down-regulation is associated with increased longevity in model organisms, was strongly associated with hypertension and stroke in an admixed population. A second population showed a trend for these associations. Furthermore, highly prevalent \textit{MAT1A} genotypes (of individual SNPs or combined as haplotypes) showed a strong interaction with plasma vitamin B-6 concentrations influencing DNA damage. Taken together, these observations imply that improving B vitamin status may decrease CVD risk in a large subset of the population, depending on \textit{MAT1A} genotype. This hypothesis can and should be tested further.

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