**MAT1A** variants are associated with hypertension, stroke, and markers of DNA damage and are modulated by plasma vitamin B-6 and folate $^{1-3}$

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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. *Am J Clin Nutr* 2010;91:1377–86.

**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-

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good in vivo data in animal models (15, 16) and in humans (17–19) suggest that changes in methylation, SAM, or methionine metabolism may be associated with CVD independently of homocysteine (16–20). Hence, genetic, nutritional, or physiologic 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. Methionine adenosyltransferase genes have been identified as a phylogenetically 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 cohort 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 approved 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 demographic 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 population was reported previously (27).

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 independent 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 during 2003–2005 (28).

Data collection and variable definition

Anthropometric measurements were collected by using standard methods. Hypertension was identified on the basis of high systolic (≥140 mm Hg) or diastolic (≥90 mm Hg) blood pressure, from the average of the latter of 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 questions: “Has a doctor ever told you that you had a stroke?” and “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 Paffenbarger questionnaire of the Harvard Alumni Activity Survey (30). Fasting blood samples were drawn by a certified phlebotomist. 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 fromuffy 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_A142A</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_i15752</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.1341–11G→A</td>
<td>15,752</td>
<td>Intron 8</td>
<td>0.20 (0.01)</td>
<td>—</td>
</tr>
<tr>
<td>MAT1A_i1262</td>
<td>rs3851059</td>
<td>NT_030059.12g.779174G→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).

falls into different LD blocks and, together, these blocks are within MAT1A and span ~10 kbp to either side of the gene.

**Linkage disequilibrium and haplotype analysis**

Pairwise LD among SNPs were estimated as correlation coefficients ($r^2$) by using the HelixTree program (Golden Helix, Bozeman, MT). For haplotype analysis, we estimated haplotype frequencies by using the Expectation-Maximization (EM) algorithm (37). To assess the association between individual haplotypes and hypertension and stroke or DNA damage, logistic or linear regression models were used while adjusting for potential confounders [methylene tetrahydrofolate reductase (MTHFR) genotype, age, sex, smoking, alcohol intake, physical activity, population admixture, medication use for diabetes and depression, and plasma folate, vitamin B-6, and vitamin B-12]. To determine the global association between haplotypes and phenotypes, we used haplotype trend regression analysis with the option of composite haplotype estimation implemented in HelixTree (38). P values were further adjusted for multiple tests with a permutation test. Because of a small population size (based on our power calculation and stratified by European and African Americans) in the NAME Study and no 8-OHdG measures in this study, an analysis of haplotypes and gene-diet interactions was not conducted in this population.

**Population admixture**

Puerto Ricans are genetically heterogeneous and originated from 3 ancestral populations: European settlers, native Taíno Indians, and West Africans. Population admixture had been evaluated previously in the BPRHS cohort by using STRUCTURE 2.2 (39) based on 100 SNPs selected as ancestry informative markers specifically for the Puerto Rican population (40). Using the estimated admixture of each participant, we adjusted for population admixture in all statistical analyses.

**Statistical analysis**

Statistical analyses were performed by using SAS 9.1 (SAS Institute Inc, Cary, NC). We assessed the relation between MAT1A variants and hypertension, stroke, CVD, and secondary characteristics, such as plasma homocysteine, systolic and diastolic blood pressures, and the urinary 8-OHdG concentration (an indicator of DNA damage). For dichotomous measures, such as stroke, hypertension, and CVD, we used a logistic regression. Continuous variables, such as DNA damage and homocysteine, were Box-Cox transformed (41) if they were not normally distributed, before general linear regression analysis. Analyses were adjusted for potential confounders (MTHFR genotype, age, sex, smoking, alcohol intake, physical activity, population admixture, medication use for diabetes and depression, and plasma folate, vitamin B-6, and vitamin B-12). Men and women were analyzed together, as well as separately, to account for possible sex-specific effects. A P value ≤0.05 was considered statistically significant. When examining genotype by nutrient interaction, we categorized participants into 2 subgroups, based on the population mean of the plasma vitamin concentrations.

**RESULTS**

**Clinical characteristics of populations and genetic variants at MAT1A**

Men and women in the BPRHS sample had a similar mean age (~58 y) and a high prevalence of diabetes (41.2% and 40.5%), hypertension (71.7% and 69.9%), and self-reported CVD (24.1% and 20.1%), respectively (Table 2). No significant difference was observed across sex for blood pressure or physical activity. Mean body mass index (BMI; in kg/m²) was significantly higher in women (33.0) than in men (29.6), whereas smoking and alcohol consumption were more prevalent in men than in women (33.8% compared with 20.3% and 49.5% compared with 35.9%, respectively). Plasma concentrations of vitamins B-6 and B-12 were similar between men and women. However, women had higher plasma folate concentrations (20.1 compared with 17.8 ng/mL) than men and tended to have more DNA damage (143 vs 124 ng 8-OHdG/mg creatinine), although the difference was not statistically significant.

In the NAME population, participants were, on average, ~18 y older than those in the BPRHS. The NAME Study population had a much higher prevalence of age-related diseases, consistent with recruitment from recipients of home-services agencies:
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>Age (y)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Diastolic blood pressure (mm Hg)</th>
<th>Plasma creatinine (mg/L)</th>
<th>Plasma folate (ng/mL)</th>
<th>Physical activity score</th>
<th>BMI (kg/m²)</th>
<th>Plasma homocysteine (µmol/L)</th>
<th>Plasma vitamin B-6 (nmol/L)</th>
<th>Plasma 8-OHdG (ng/mg creatinine)</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Smoker</th>
<th>Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>57.4 ± 7.8</td>
<td>56.7 ± 7.3</td>
<td>74.1 ± 8.3</td>
<td>75.2 ± 8.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>29.6 ± 5.1</td>
<td>33.0 ± 6.9</td>
<td>28.6 ± 6.0</td>
<td>32.4 ± 9.1</td>
<td>0.037</td>
<td>0.137</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137.7 ± 18.4</td>
<td>155.3 ± 18.8</td>
<td>137.0 ± 21.0</td>
<td>134.5 ± 20.0</td>
<td>0.673</td>
<td>0.414</td>
<td>82.7 ± 11.2</td>
<td>80.5 ± 10.3</td>
<td>76.3 ± 11.8</td>
<td>75.3 ± 10.7</td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>32.5 ± 5.7</td>
<td>31.1 ± 4.0</td>
<td>26.9 ± 1.4</td>
<td>26.8 ± 1.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>17.8 ± 8.6</td>
<td>20.1 ± 9.3</td>
<td>15.5 ± 12.3</td>
<td>15.0 ± 8.9</td>
<td>0.011</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>529.9 ± 271.3</td>
<td>546.8 ± 278.4</td>
<td>519.9 ± 262.9</td>
<td>607.4 ± 546.7</td>
<td>0.655</td>
<td>0.005</td>
<td>61.6 ± 59.5</td>
<td>60.0 ± 64.1</td>
<td>69.7 ± 73.8</td>
<td>69.9 ± 79.4</td>
<td>0.151</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.7 ± 5.9</td>
<td>8.7 ± 4.1</td>
<td>12.4 ± 4.9</td>
<td>11.9 ± 5.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>9.8 ± 3.8</td>
<td>7.7 ± 3.5</td>
<td>11.9 ± 10.0</td>
<td>10.6 ± 10.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-OHdG (ng/mg creatinine)</td>
<td>124.2 ± 69.2</td>
<td>143.1 ± 75.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>99 (33.8)</td>
<td>142 (20.3)</td>
<td>67 (24.8)</td>
<td>131 (15.3)</td>
<td>0.018</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker [n (%)]</td>
<td>146 (49.5)</td>
<td>254 (35.9)</td>
<td>100 (37.2)</td>
<td>276 (32.1)</td>
<td>&lt;0.001</td>
<td>122 (41.2)</td>
<td>285 (40.5)</td>
<td>112 (42.0)</td>
<td>292 (35.2)</td>
<td>0.846</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinker [n (%)]</td>
<td>210 (71.7)</td>
<td>490 (69.9)</td>
<td>226 (84.3)</td>
<td>718 (85.2)</td>
<td>&lt;0.001</td>
<td>16 (5.4)</td>
<td>23 (3.3)</td>
<td>66 (24.7)</td>
<td>163 (19.2)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension [n (%)]</td>
<td>72 (24.1)</td>
<td>142 (20.1)</td>
<td>117 (43.8)</td>
<td>344 (40.9)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 NA; not applicable; 8-OHdG, 8-hydroxodeoxyguanosine.
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 vs. men (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 (rs70877728) 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 A 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 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).
Interaction of 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 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 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 MAT1A SNP on 8-OHdG concentrations in this population (data not shown).

Association between MAT1A haplotypes, stroke, hypertension, CVD, and DNA damage

To examine the combined effect of MAT1A genetic variants on stroke, hypertension, CVD, and DNA damage in the BPRHS, we performed haplotype analysis based on 3 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 (i.e., 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, 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. 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 MAT1A haplotypes and CVD did not reach global significance (data not shown). Interestingly, when heart attack was excluded from CVD, 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 MAT1A haplotypes and homocysteine and DNA damage showed that 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 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**

*MATIA* 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 *MATIA* 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 *MATIA* 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 *MATIA* variation with stroke and hypertension is independent of *MTHFR*.

Our results indicate that the direction of the interaction between *MATIA* 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 *MATIA* genotype showed a strong interaction with vitamin B-6 on DNA damage suggests that *MATIA* genotype may influence the potential for cardiovascular protection through an improvement in vitamin B-6 status. Such potential confounding by *MATIA* 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 marginal so, to conclude that vitamin B-6 was responsible for the observed differences.

**TABLE 4**

| Frequency | Odds ratio | 95% CI | P value | Frequency | Odds ratio | 95% CI | P value | Frequency | Odds ratio | 95% CI | P value |
|-----------|------------|--------|---------|-----------|------------|--------|---------|---------|-----------|--------|---------|---------|
| G-G-G     | 0.37       | 1.62   | 0.77    | 3.42      | 0.203      | 1.31   | 0.94    | 1.81     | 0.109     | 1.31   | 0.88    | 1.93     | 0.174   |
| A-G-A     | 0.27       | 0.84   | 0.40    | 1.76      | 0.648      | 1.00   | 0.72    | 1.38     | 0.975     | 1.01   | 0.69    | 1.48     | 0.975   |
| A-A-G     | 0.26       | 0.27   | 0.11    | 0.65      | 0.003      | 0.63   | 0.46    | 0.87     | 0.005     | 0.58   | 0.39    | 0.86     | 0.007   |
| A-G-G     | 0.07       | 0.46   | 0.10    | 0.20      | 0.310      | 0.92   | 0.53    | 1.57     | 0.752     | 0.73   | 0.36    | 1.47     | 0.380   |
| G-G-A     | 0.03       | 1.83   | 0.51    | 6.61      | 0.358      | 1.24   | 0.59    | 2.57     | 0.572     | 2.30   | 1.15    | 4.60     | 0.018   |

1. *MATIA* haplotypes were associated with stroke at a global significance of *P* = 0.001, or *P* = 0.002 after a permutation test.
2. *MATIA* haplotypes were associated with hypertension at a global significance of *P* = 0.012, or *P* = 0.020 after a permutation test.
3. *MATIA* haplotypes were associated with CVD (heart attack was excluded) at a global significance of *P* = 0.018, or *P* = 0.025 after a permutation test.
4. *MATIA* haplotypes were estimated based on 3 single nucleotide polymorphisms in the following order: i15173, 3U1510, and d18777.
5. *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.

**TABLE 5**

| Frequency | Odds ratio | 95% CI | P value | Frequency | Odds ratio | 95% CI | P value | Frequency | Odds ratio | 95% CI | P value |
|-----------|------------|--------|---------|-----------|------------|--------|---------|---------|-----------|--------|---------|---------|
| G-G-G     | 0.37       | 1.62   | 0.77    | 3.42      | 0.203      | 1.31   | 0.94    | 1.81     | 0.109     | 1.31   | 0.88    | 1.93     | 0.174   |
| A-G-A     | 0.27       | 0.84   | 0.40    | 1.76      | 0.648      | 1.00   | 0.72    | 1.38     | 0.975     | 1.01   | 0.69    | 1.48     | 0.975   |
| A-A-G     | 0.26       | 0.27   | 0.11    | 0.65      | 0.003      | 0.63   | 0.46    | 0.87     | 0.005     | 0.58   | 0.39    | 0.86     | 0.007   |
| A-G-G     | 0.07       | 0.46   | 0.10    | 0.20      | 0.310      | 0.92   | 0.53    | 1.57     | 0.752     | 0.73   | 0.36    | 1.47     | 0.380   |
| G-G-A     | 0.03       | 1.83   | 0.51    | 6.61      | 0.358      | 1.24   | 0.59    | 2.57     | 0.572     | 2.30   | 1.15    | 4.60     | 0.018   |

1. *MATIA* haplotypes were estimated based on 3 single nucleotide polymorphisms in the following order: i15173, 3U1510, and d18777.
2. *P* values were calculated by linear regression models and adjusted 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).
3. Mean; SE in parentheses (all such values).
4. The interaction between haplotype A-A-G and vitamin B-6 was highly significant at *P* = 0.0004, whereas there was no significant interaction between other haplotypes and vitamin B-6.
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 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 (VISP) (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 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 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. 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 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 MAT1A genotype, which is consistent with previous observations (1).

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The authors’ responsibilities were as follows—C-QL, JMO, and KLT: study concept and design; C-QL, JS, JC, Y-CL, and HC: acquisition of data; 1384 LAI ET AL
C-QL, LDP, AMT, DW, and KLT: analysis and interpretation of data; C-QL: drafting of the manuscript; C-QL, LDP, AMT, WQQ, IHR, and KLT: critical revision of the manuscript for intellectual content; C-QL: statistical analysis; and JMO, KLT, and IHR: funding and supervision. None of the authors had a conflict of interest.

REFERENCES


