The Journal of Nutrition
Nutritional Epidemiology

Status of Vitamins B-12 and B-6 but Not of Folate, Homocysteine, and the Methylenetetrahydrofolate Reductase C677T Polymorphism Are Associated with Impaired Cognition and Depression in Adults[1–3]

Denish Moorthy,[4,13] Inga Peter,[9] Tammy M. Scott,[4,10] Laurence D. Parnell,[5] Chao-Qiang Lai,[5] Jimmy W. Crott,[6] José M. Ordovás,[5] Jacob Selhub,[7] John Griffith,[11] Irwin H. Rosenberg,[4] Katherine L. Tucker,[8] and Aron M. Troen[4,12]*

[4]Neuroscience and Aging Laboratory, [5]Nutritional Genomics Laboratory, [6]Vitamins and Carcinogenesis Laboratory, [7]Vitamin Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; [8]Department of Health Sciences, Northeastern University, Boston MA; [9]Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine, New York, NY; [10]Department of Psychiatry, and [11]Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, MA; and [12]Institute of Biochemistry, Food Science and Nutrition, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Abstract

The C677T polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene differs in frequency in various ethnic groups that have differing prevalence of age-related cognitive impairments. We used a series of neuro-psychological tests to examine the association of the *MTHFR* C677T polymorphism with cognition and depression and also to assess whether genotype modifies the association of folate and homocysteine with these outcomes. This study analyzed pooled cross-sectional data from 2 ethnically diverse cohorts of community-living adults: the Boston Puerto Rican Health Study ($n = 939$) and the Nutrition, Aging, and Memory in Elders study ($n = 1017$). Individuals in both cohorts underwent anthropometric and laboratory measurements and dietary and health assessments using validated questionnaires between the years 2003 and 2007. Cognitive outcomes included measures of global cognition [Mini-Mental Status Exam (MMSE)], depression (Center for Epidemiological Studies Depression Scale), and 3 factor scores for the domains of attention, executive function, and memory that were derived from a detailed set of neuropsychological tests. Low plasma vitamin B-12 concentrations were associated with poorer MMSE scores and higher depression scores, and low vitamin B-6 concentrations were associated with lower MMSE and worse attention and executive function in the multivariate analysis. In contrast, *MTHFR* genotype, folate, and homocysteine were not associated with cognition or depression in either ethnicity-pooled or stratified analysis. The current study did not find evidence of an association between the *MTHFR* C677T TT genotype and impaired cognition or depression in a population with adequate folate status and a high prevalence of cognitive impairment and depression. J. Nutr. 142: 1554–1560, 2012.

Introduction

Cognitive decline with age is attributable to both genetic and environmental factors. Modifiable environmental factors, including nutrition, may hold the key to prevention of age-associated cognitive impairment (1). Elevated plasma total homocysteine (tHcy)[14] concentration is one of the most widely studied potentially modifiable risk factors. Elevated tHcy and low B-vitamin concentrations have been associated with various measures of poor cognitive outcomes in 90 of 100 cross-sectional and prospective studies encompassing a total of 46,801 men and women (2). In the Framingham Heart Study, a 5-μmol/L increase in tHcy was estimated to increase the risk of incident

[1] Supported by a pilot grant to AMT from the Ross Institute of Aging, NIH POI AG023394, NIA; RO1 AG21790; NIH MO1-RR00054 and USDA (under cooperative agreement no. 58-1950-9-001).
[2] Author disclosures: D. Moorthy, I. Peter, T. M. Scott, L. D. Parnell, C. Lai, J. W. Crott, J. M. Ordovás, J. Selhub, J. Griffith, I. H. Rosenberg, K. L. Tucker, and A. M. Troen, no conflict of interest.
[3] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[13] Present address: Senior Research Associate, Center for Clinical Evidence Synthesis, Institute for Clinical Research and Health Policy Studies, Tufts Medical Center, Boston, MA 02111. E-mail: DMoorthy@tuftsmedicalcenter.org.
* To whom correspondence should be addressed. E-mail: Troen@agri.huji.ac.il.

[14] Abbreviations used: BPRHS, Boston Puerto Rican Health Study; CES-D, Center for Epidemiological Studies Depression Scale; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Exam; NAME, Nutrition, Aging, and Memory in Elders; SNP, single nucleotide polymorphism; tHcy, plasma total homocysteine.

Manuscript received March 23, 2012. Initial review completed April 14, 2012. Revision accepted May 26, 2012.
First published online June 27, 2012; doi:10.3945/jn.112.161828.

Alzheimer's disease by 40%, which is similar in magnitude to the genetic risk conferred by carrying the apoE E4 allele (3). Consistent with these associations, elevated tHcy is also prospectively associated with brain atrophy (4,5). Moreover, tHcy has been hypothesized to play a significant role in the pathogenesis of depression (6), although this association is less well established, with studies showing both increased (7) and decreased (8) risk of depression in individuals with higher tHcy concentrations.

Collectively, these associations are the basis of the plausible hypothesis that elevated tHcy causes age-associated cognitive decline and that therefore, lowering tHcy may mitigate decline. This hypothesis has yet to be conclusively upheld or ruled out by randomized clinical trials (9). However, whereas the majority of randomized clinical trials on homocysteine lowering have been negative (10–13), 2 trials found clear improvement in memory and cognition upon folic acid supplementation, both alone and in combination with vitamins B-12 and B-6 (14,15). Within the negative trials, one trial (12) reported a lower Alzheimer Disease Assessment Scale score after 15 mo of treatment compared with placebo in those who had a baseline Mini-Mental State Exam (MMSE) score above the group median, which indicates a differential effect of treatment depending on baseline cognitive status.

Gene-nutrient association studies could shed light on the apparent discordance between the observational studies and trials and help to identify groups and individuals who might benefit from treatment. On this basis, several studies have explored the relationship between a prevalent single nucleotide polymorphism (SNP) of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*, a key enzyme of folate metabolism) gene, C677T, with homocysteine, cognition, and depression. The *MTHFR* C677T variant encodes a thermo-labile enzyme with lower activity (16), which alters the balance of nucleotide synthesis, homocysteine clearance, and methionine synthesis in homozygous carriers of the *T* allele. TT genotype has been reportedly associated with higher tHcy concentrations compared with the CC or CT genotypes (17). This difference in tHcy is most pronounced at low folate concentrations, because folate binding stabilizes the enzyme (18,19). If high tHcy is harmful to the brain, then individuals with the TT genotype, who have a lifetime exposure to higher homocysteine, should be at higher risk of cognitive impairment than CC and CT individuals. Moreover, the risk should be most pronounced in individuals with low habitual folate intake.

Studies of the association between the *MTHFR* C677T SNP, tHcy, and cognition have been inconsistent, with some reporting no relationship between the *MTHFR* C677T variant and cognition (20–23) and others reporting domain- or test-specific associations (24–27). Similar inconsistencies are seen for the association between *MTHFR* and depression (28–31). Discrepancies between studies may be due to 2 important factors: *1*) with the exception of the study by Elkins et al. (27), none of the reported studies addressed potential confounding by ethnicity (21,32,33); and *2*) differences in folate status. We therefore examined the associations of the *MTHFR* C677T polymorphism with performance in discrete cognitive domains and with depression, and in relation to plasma folate and tHcy concentrations in 2 community cohort studies consisting of 3 ethnically diverse populations, using a battery of neuropsychiatric tests.

Participants and Methods

Participants and study design. We conducted the current cross-sectional study in 2 Boston-based cohorts: the Boston Puerto Rican Health Study (BPRHS) and the Nutrition, Aging, and Memory in Elders (NAME) study, which are described in detail elsewhere (34,35). BPRHS consists of Boston area residents of Puerto Rican origin aged 45–75 y. The NAME study is a cohort of community-based, African American, and non-Hispanic white elderly participants aged 60 y and older recruited through Boston area Aging Services Access Points, which are nonprofit agencies that provide information and a wide range of services to the elderly for their care. Both cohorts were administered a health questionnaire, an FFQ, neuropsychological tests, and anthropometric measures and provided blood for laboratory measurements. Overall, 1017 and 938 participants of the BPRHS and NAME cohorts, respectively, were genotyped for this study, providing a total sample size of 1955. Informed consent for all tests, including genotyping, was obtained. Approval for the BPRHS and NAME was obtained from the Institutional Review Board of the Tufts Medical Center and Tufts University.

DNA isolation and genotyping. DNA was isolated using QIAamp DNA Blood Mini kits (Qiagen). The MTHFR C677T SNP (rs1801133, chromosome 1, exon 5) was genotyped with Applied Biosystems TaqMan SNP genotyping system (36). For all genotyping, blinded, no-template controls and replicates of DNA samples were incorporated in each of the DNA sample plates, which were routinely checked by laboratory personnel. *ApoE* was genotyped as described elsewhere (37).

Primary outcome: cognition. Assessment of cognition in both cohorts was supervised by the same research team and neuropsychologist. Global cognition was tested using the MMSE (38) and depression was assessed using the Center for Epidemiological Studies Depression Scale (CES-D) (39). Cognition was tested using a battery of sensitive, standardized, and normed neuropsychological tests (**Supplemental Table 1**). For each participant, component scores representing the cognitive domains of memory, executive function, and attention were postestimated following a principal component analysis of selected neuropsychological test scores (40–42).

Covariates. General demographic information such as age, sex, ethnicity, and education, and health history and behaviors, including chronic disease history, was elicited by questionnaire. Kidney function was evaluated by calculating the estimated glomerular filtration rate (eGFR) from serum creatinine using the Modification of Diet in Renal Disease equation (43). *ApoE* genotype was classified as carriers of the E4 allele (E3E4 and E4E4) and others (E3E3, E2E3, E2E2). Individuals with the E2E4 genotype were excluded from the analysis as per convention.

Biochemical measurements. Nutritional predictors included plasma concentrations of folate, vitamins B-12 and B-6, and tHcy. tHcy was determined by HPLC (44). The plasma creatinine concentration was measured by a modified Jaffe method (45). Plasma folate and vitamin B-12 were measured with an Immulite chemiluminescent assay (Diagnostic Products/Siemens). Pyridoxal 5-phosphate (vitamin B-6) was determined by the tyrosine decarboxylase apoenzyme method (46).

Statistical analysis. The observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium using a χ^2 test. Based on known functional effects, we used the recessive mode of inheritance where carriers of the 2 *MTHFR T* alleles (TT genotype) were compared with those who carry one or zero *T* alleles (CT or CC genotypes). Initial analyses were carried out on the pooled data from the 2 cohorts. Linear regression was performed to detect associations of the MMSE, CES-D, cognitive factor scores, and logarithmically transformed homocysteine concentration with *MTHFR* status. For the MMSE, we used a Tobit regression model (47), because the MMSE is a variable that is right truncated, with a maximum score of 30. Adjustments were made for age, sex, ethnicity, and education as well as *ApoE4* genotype, plasma folate, vitamins B-12 and B-6, kidney function, and presence of diabetes and hypertension. Given that folate and vitamins B-12 and B-6 are metabolic determinants of tHcy and that there are several plausible mechanisms for the inverse association between tHcy and cognition that may be either dependent or independent of B vitamin status, models that

include all the variables make the statistical assumption that significant associations between any given variable and tHcy represent residual independent effects. However, it is equally possible that including B vitamins and tHcy in the same model can overfit the model. Therefore, a sensitivity analysis of the final regression model was carried out by dropping the terms for the B vitamins. In addition, we carried out race-stratified analyses by 3 self-identified categories: African American or non-Hispanic white participants of the NAME study cohort and Puerto Rican participants of the BPRHS cohort. We assessed gene-nutrient interactions by including a cross product term for folate and SNP genotypes in the linear regression model. All of the analyses were performed using SAS/STAT 9.1.3 (SAS Institute). A 2-sided $P < 0.05$ was considered significant.

Results

Compared with the BPRHS cohort, NAME participants were older, better educated, and had a higher proportion of women (**Table 1**). Both populations had a high prevalence of chronic conditions, including hypertension and diabetes. Among African Americans and Puerto Ricans, there was a higher prevalence of cognitive impairment, as measured by MMSE (less than the cutoff of 24–55.9% in African Americans, 61.9% in Puerto Ricans, and 29.7% in non-Hispanic whites; overall $P < 0.001$). Puerto Ricans also had a higher prevalence (60.5%) of elevated depressive symptoms (CES-D $\geq$ 16) than African Americans (34.7%) and non-Hispanic whites (33.8%) (overall $P < 0.001$).

The distribution of the variant *T* allele differed among the 3 population groups (minor allele frequencies of 12.5 and 37.9% for African Americans and non-Hispanic whites, respectively, and 34.9% for Puerto Ricans), with genotypes conforming to the Hardy-Weinberg equilibrium in each subpopulation. The distribution of *ApoE* E4 alleles did not significantly differ among the *MTHFR* genotypes.

The prevalence of cognitive impairment and depressive symptoms did not significantly differ between those with the TT genotype compared with the CC+CT genotype in any ethnic group (Table 1).

NAME participants had a significantly higher mean tHcy concentration (12.5 ± 6.2 and 11.7 ± 4.5 μmol/L for African Americans and non-Hispanic whites, respectively) compared with Puerto Ricans (9.2 ± 4.7 μmol/L) and a lower folate concentration (31.5 ± 23.6 and 36.0 ± 20.6 nmol/L for African Americans and non-Hispanic whites, respectively) compared with Puerto Ricans (43.5 ± 19.9 nmol/L). Nevertheless, the prevalence of low folate concentrations (defined as <9 nmol/L) was very low (1.6% in African Americans, 2.0% in non-Hispanic whites, and 0.2% in Puerto Ricans). When further stratified by the *MTHFR* C677T genotype, those with the TT genotype had a mean 3.6 nmol/L lower folate concentration than the CC+CT group, but this was not significant. Carriers of the TT genotype among African Americans and non-Hispanic whites also tended to have lower vitamin B-12 concentrations compared with the CC+CT genotypes, but this difference was not significant ($P < 0.10$). However, among Puerto Ricans, the TT group had higher vitamin B-12 concentrations than the CC +CT group ($P < 0.05$) (Table 1).

In the pooled analysis controlling for ethnicity, tHcy was higher in TT carriers relative to the CC and CT genotypes, but this difference was not significant. In the stratified analysis, tHcy was significantly higher among African American and non-

TABLE 1 Description of the NAME and BPRHS populations[1]

	NAME				BPRHS	
	African Americans		Non-Hispanic whites		Puerto Ricans	
	TT	CT + CC	TT	CT + CC	TT	CT + CC
n	9	363	102	543	116	823
Age, *y*	69.0 ± 6.5	74.1 ± 8.2	75.0 ± 8.7	76.3 ± 8.5	56.9 ± 7.4	58.0 ± 7.3
Female, %	100	81.3	70.6	72.2	64.2 *	72.9*
Education, %						
0–11th grade	11.1	47.7	30.4	26.2	67.0	67.0
12th grade/high school graduate	44.4	28.5	31.4	36.9	20.0	18.2
Some college/bachelor's degree	33.3	21.6	33.3	31.5	12.1	12.5
Graduate school	11.1	2.2	4.9	5.4	0.9	2.3
MMSE score	23.9 ± 1.1	23.8 ± 0.2	25.8 ± 0.3	25.8 ± 0.1	23.7 ± 0.3	23.3 ± 0.1
MMSE score <24, %	44.4	54.8	30.4	27.8	49.6*	64*
CES-D depression score	18.0 ± 3.5	13.5 ± 0.6	12.6 ± 1.1	12.8 ± 0.4	20.1 ± 1.2†	20.4 ± 0.5†
CES-D score ≥16, %	44.4	34.2	31.9	34.0	60†	60.9†
eGFR,[2] $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$	93.0 ± 52.8	85.4 ± 36.6	81.8 ± 33.0	81.4 ± 33.7	88.3 ± 23.7	87.3 ± 23.6
Folate, *nmol/L*	24.5 ± 7.9	32.0 ± 1.4	33.5 ± 2.0	36.5 ± 0.9	43.1 ± 2.0	44.0 ± 0.7
Vitamin B-12,[2] *pmol/L*	340 ± 190	504 ± 30	366 ± 21	407 ± 9	439 ± 19*	394 ± 7*
Vitamin B-6,[2] *nmol/L*	33.8 ± 19.8	57.9 ± 3.1	75.0 ± 8.6	74.9 ± 3.7	58.8 ± 5.9	59.6 ± 2.2
tHcy,[2] *μmol/l*	16.8 ± 2.1*	12.4 ± 0.3*	12.5 ± 0.4*	11.6 ± 0.2*	9.7 ± 0.4	9.2 ± 0.2
Diabetes, %	55.6	44.9	34.3	29.3	43.5	42.0
Hypertension, %	77.8	91.3	81.0	82.8	73.9	70.4
apoE4 genotype, %	22.2	28.5	18.2	17.1	28.0	24.4

[1] Values are estimated means ± SE; pairwise comparisons flagged as significant are between TT and CC+CT genotypes within separate ethnic subgroups: *$P < 0.05$, **$P < 0.01$, †$P < 0.001$. One-way ANOVA and chi-square tests were used to test significance in the 3 population groups. BPRHS, Boston Puerto Rican Health Study; CES-D, Center for Epidemiological Studies Depression Scale; eGFR, estimated glomerular filtration rate; MMSE, Mini-Mental State Exam; NAME, Nutrition, Aging, and Memory in Elders; tHcy, plasma total homocysteine.

[2] Comparisons adjusted for age, sex, and education.

Hispanic white TT carriers than in the CC+CT group, by 4.4 and 0.9 μmol/L, respectively (Table 1). In multivariate regression analysis, neither *MTHFR* C677T TT genotype nor tHcy were associated with cognition or depression (**Table 2**). Low vitamin B-12 was associated with poorer MMSE scores and higher depression scores, and low vitamin B-6 was associated with lower MMSE and worse attention and executive function in the multivariate analysis (Table 2). Because race was an important predictor of these outcomes, we repeated the regression model in each of the 3 ethnic subgroups. On stratification, *MTHFR* TT was not associated with cognition or depression in any of the 3 subgroups. In the interaction model, the *MTHFR* TT genotype showed no effect modification by folate in predicting the cognitive outcomes or depression, either in the pooled analysis or in the stratified analysis. The interaction term for race and *MTHFR* genotype was not significant (data not shown). Further, we conducted a sensitivity analysis by excluding B vitamins from the model and that did not result in changes in the effect of homocysteine on cognition. The only exception being that when B vitamins were excluded from the model, homocysteine became a marginally significant predictor of the executive function score (i.e., $P = 0.02$ is considerably greater than the $P < 0.005$ required for significance after adjustment for multiple testing). Nevertheless, the difference in the regression coefficient when B vitamins were included or omitted from the model was only 0.02 units ($\beta = -0.15$, $P = 0.051$; and $\beta = -0.17$, $P = 0.02$, respectively). This change in significance is marginal, considering that the sensitivity analysis involved multiple testing of the association of tHcy with 5 cognitive outcomes (MMSE, CES-D, executive function, attention, and memory), both with and without B vitamins. Moreover, the difference between the 2 models is subtle and difficult to interpret, considering that the strength of the association was relatively weak, and no association was found between tHcy and any of the other cognitive outcome measures. Although we cannot rule out a biologically real association, the β coefficients for the B vitamins remain basically unchanged with regard to the magnitude and direction of the effect and significance (Table 2).

Whereas diabetes, hypertension, and *ApoE* genotype were associated with various cognitive outcomes, eGFR was not a significant predictor of either tHcy or cognition in our study.

Discussion

Lifelong "exposure" to the *MTHFR* C667T TT genotype results in modest increases in circulating tHcy concentrations and altered folate metabolism, although this phenotype can be mitigated by improving folate status. If the hypothesis that elevated tHcy concentrations and poor folate status cause age-associated cognitive decline is correct, then the *MTHFR* TT genotype would be predictive of poorer cognitive outcomes compared with the CC and CT genotypes, particularly among individuals with poor folate status. Consistent with this hypothesis, a few studies have reported an association between the *MTHFR* TT genotype and increased risk of Alzheimer's disease (48,49). Testing this hypothesis is important to identify key genetic or ethnic characteristics of those individuals who may benefit the most from folate supplementation to lower tHcy concentrations and prevent age-associated cognitive decline.

We therefore determined whether the *MTHFR* C677T variant was associated with impaired cognition or depression independently or through its tendency to elevate tHcy in 2 cross-sectional, multi-ethnic cohorts: the BPRHS, composed of individuals of Puerto Rican descent, and the NAME study, composed of non-Hispanic whites and African Americans. We also assessed whether the effect of this genotype on cognitive function might be modified by folate.

In contrast to the prediction, we did not find *MTHFR* C677T to be associated with impaired cognition or depression, nor did folate modify the relationship of the *MTHFR* C677T TT genotype with impaired cognition and depression. Furthermore,

TABLE 2 Parameter estimates and SE from the multivariate linear regression of cognitive outcomes and depression in the pooled dataset[1]

Variables	MMSE (n = 1766)	CES-D (n = 1677)	FACattn (n = 1532)	FACexec (n = 1532)	FACmem (n = 1532)
	β (SE)				
MTHFR TT genotype	0.06 (0.24)	0.18 (0.91)	−0.05 (0.08)	0.06 (0.07)	0.06 (0.08)
Ethnicity: African American[2]	0.58 (0.29)*	−2.28 (1.09)*	−0.37 (0.09)†	0.1 (0.09)	0.36 (0.09)†
Ethnicity: non-Hispanic whites[2]	2.34 (0.28)†	−2.13 (1.04)*	−0.22 (0.09)*	0.71 (0.08)†	0.31 (0.09)†
Age	−0.07 (0.01)†	−0.25 (0.04)†	0.01 (0.003)*	−0.03 (0.003)†	−0.02 (0.003)†
Sex	−0.33 (0.18)	3.36 (0.66)†	−0.2 (0.06)†	0.08 (0.05)	0.33 (0.06)†
Education	1.03 (0.06)†	−0.94 (0.23)†	0.2 (0.02)†	0.22 (0.02)†	0.1 (0.02)†
apoE E4 genotype	0.04 (0.18)	−0.23 (0.67)	−0.02 (0.06)	0.01 (0.05)	−0.09 (0.06)
eGFR	0.001 (0.003)	−0.02 (0.01)	−0.0004 (0.001)	0.0002 (0.001)	0.0003 (0.001)
Diabetes	−0.43 (0.16)**	0.25 (0.61)	−0.12 (0.05)*	−0.15 (0.05)**	−0.08 (0.05)
Hypertension	0.48 (0.19)*	0.59 (0.73)	0.09 (0.06)	−0.02 (0.06)	0.09 (0.06)
tHcy[3]	0.23 (0.26)	−1.05 (0.96)	−0.03 (0.08)	−0.15 (0.08)	0.03 (0.08)
Folate[3]	−0.09 (0.17)	−0.8 (0.65)	0.05 (0.06)	−0.02 (0.05)	0.09 (0.06)
Vitamin B-12[3]	0.41 (0.17)*	−1.65 (0.65)*	0.02 (0.06)	−0.03 (0.05)	0.07 (0.06)
Vitamin B-6[3]	0.28 (0.12)*	−0.57 (0.43)	0.11 (0.04)**	0.08 (0.03)*	−0.04 (0.04)

[1] Adjusted as described in Methods. β (SE) = parameter estimate (SE) *$P < 0.05$, **$P < 0.01$, †$P < 0.001$. CES-D, Center for Epidemiological Studies Depression Scale; eGFR, estimated glomerular filtration rate; FACattn, factor score for attention; FACexec, factor score for executive function; FACmem, factor score for memory; MMSE, Mini-Mental State Exam; tHcy, plasma total homocysteine.
[2] Compared with Puerto Rican.
[3] Plasma analytes were log-transformed.

tHcy concentration was not associated with cognition or depression in our study populations, even though tHcy concentrations were significantly higher among African American and non-Hispanic white carriers of the *MTHFR* TT genotype. Nevertheless, the prevalence of folate deficiency in our study was very low, presumably through exposure to mandatory food folic acid fortification and supplement use and thus the absolute effect of the TT genotype on elevating tHcy concentrations was modest.

Other studies also have not detected an association between *MTHFR* C677T genotype and neurocognitive outcomes, even in populations not exposed to fortification (50,51). For example, genotype did not predict brain lesions in the Rotterdam Brain Scan Study, where tHcy concentrations were comparable with the present study (12 μmol/L) and folate status was worse (median: 12.0 nmol/L) (20). Indeed, in another Dutch population, individuals with the TT genotype performed better on multiple cognitive tests compared with participants with the CC or CT genotype (32). Moreover, folate status has been reported to be associated with cognition and depression, independent of tHcy, even in populations exposed to food folate fortification (52,53). Thus, it is difficult to dismiss our findings as solely reflecting a modifying effect of adequate folate status in our population on the association between *MTHFR* C677T genotype and cognition.

A strength of the present study is the analysis of a large, ethnically diverse population in which the allele frequencies of *MTHFR* were significantly different. Most previous studies have been conducted in predominantly homogeneous Caucasian populations (27). Stratifying our analysis by ethnicity reduced confounding by population structure. The ethnic subgroups differed by age, education, blood biochemical variables (folate, vitamins B-12 and B-6, and homocysteine) and the prevalence of diabetes and hypertension. However, within each subgroup, these factors did not differ by *MTHFR* genotype. An added strength of our study was adjustment for the apoE E4 allele, which is a potential modifier of the relationship between tHcy and cognitive function (54). This was performed to control for potential confounding by the apoE E4 allele, which was done in only one other study (32).

Age might also modify the associations of *MTHFR*, folate, and tHcy status with cognition and depression (55) (19). The age variation in our study (range: 45–103 y, with 6.7% >85 y), allowed us to examine this possibility; however, we found no evidence for an effect of age on the association between *MTHFR*, folate, and tHcy status with cognition and depression.

Despite the unexpected failure to detect the predicted associations of *MTHFR* genotype, tHCy, and folate concentrations with cognition and depression, we did detect associations between lower vitamin B-12 and B-6 concentrations and poorer cognition and depression. Higher vitamin B-12 and B-6 concentrations were associated with better overall global cognition scores; vitamin B-12 was also associated with lower depression scores and vitamin B-6 with better attention and executive function scores, independent of homocysteine. Statistical independence notwithstanding, the metabolism of homocysteine, folate, and vitamins B-12 and B-6 are always closely interrelated. Nevertheless, a sensitivity analysis indicated that including all 3 B vitamins and homocysteine in the model does not lead to overfitting. Moreover, similar findings of distinct associations between vitamin B-12 and B-6 and specific cognitive domains, irrespective of homocysteine or folate, were previously reported (56,57). Such results are also consistent with other studies finding no relationship between *MTHFR* genotype and cognitive function, irrespective of tHcy (22,23,50,51).

Limitations of the present study include its cross-sectional design and limited power to detect significant gene-nutrient interactions. Although it was well powered to detect main effects of *MTHFR*, tHcy, and vitamin status, the sample size needed to reach 80% statistical power to detect significant gene-nutrient interaction ranges from several hundred to several thousand participants, depending on the outcome variable of interest. The use of different neuropsychological tests in the NAME and BPRHS populations precluded direct comparison of test scores between populations; however, the derivation of factor scores allowed the valid comparison and pooling of the different populations, lending strength to the study. Finally, because kidney dysfunction is another important determinant of tHcy (58) and cognitive risk (59), we adjusted for calculated eGFR, finding that eGFR was not a significant predictor of either tHcy or cognition in these populations.

In conclusion, our findings in an ethnically diverse population with adequate folate status and a high prevalence of cognitive impairment, depression, and comorbidity do not support the hypothesis that the *MTHFR* C677T TT genotype causes homocysteine-mediated impairment of cognition or depression. Given the small effect size of the *MTHFR* C677T polymorphism on cognition observed here and in the literature (60,61), this polymorphism is likely to be of limited clinical significance for cognition and depression, at least in folate-replete populations. If so, universal folate supplementation for neurocognitive protection is not likely to be generally effective. Nevertheless, if the reported associations between *MTHFR* genotype, tHcy, and cognition in some studies are not due to chance but rather to some other confounding factors, it will be important to identify such factors in order to intervene effectively in those individuals for whom it might be possible to mitigate the associated risk of cognitive impairment and depression.

Acknowledgments

The authors thank Yu-Chi Lee for technical assistance with genotyping. I.P., L.D.P., J.M.O., I.H.R., K.L.T., and A.M.T. designed research; D.M., T.M.S., and J.W.C. conducted research; T.M.S., L.D.P., C.L., J.W.C., J.M.O., J.S., J.G., I.H.R., and K.L.T. provided essential materials; D.M., I.P., and A.M.T. analyzed data and wrote the paper; and A.M.T. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited

1. Williams CL. Food for thought: brain, genes, and nutrition. Brain Res. 2008;1237:1–4.
2. Smith AD. The worldwide challenge of the dementias: a role for B-vitamins and homocysteine? Food Nutr Bull. 2008;29:S143–72.
3. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PWF, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. 2002;346:476–83.
4. den Heijer T, Vermeer SE, Clarke R, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MM. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain. 2003;126:170–5.
5. Seshadri S, Wolf PA, Beiser AS, Selhub J, Au R, Jacques PF, Yoshita M, Rosenberg IH, D'Agostino RB, DeCarli C. Association of plasma total homocysteine levels with subclinical brain injury: cerebral volumes, white matter hyperintensity, and silent brain infarcts at volumetric magnetic resonance imaging in the Framingham Offspring Study. Arch Neurol. 2008;65:642–9.
6. Folstein M, Liu T, Peter I, Buell J, Arsenault L, Scott T, Qiu WW. The homocysteine hypothesis of depression. Am J Psychiatry. 2007;164:861–7.

7. Almeida OP, McCaul K, Hankey GJ, Norman P, Jamrozik K, Flicker L. Homocysteine and depression in later life. Arch Gen Psychiatry. 2008; 65:1286–94.
8. Forti P, Rietti E, Pisacane N, Olivelli V, Dalmonte E, Mecocci P, Ravaglia G. Blood homocysteine and risk of depression in the elderly. Arch Gerontol Geriatr. 2010;51:21–5.
9. Nachum-Biala Y, Troen AM. B-vitamins for neuroprotection: narrowing the evidence gap. Biofactors. 2012;.
10. Eussen SJ, de Groot LC, Joosten LW, Bloo RJ, Clarke R, Ueland PM, Schneede J, Blom HJ, Hoefnagels WH, van Staveren WA. Effect of oral vitamin B-12 with or without folic acid on cognitive function in older people with mild vitamin B-12 deficiency: a randomized, placebo-controlled trial. Am J Clin Nutr. 2006;84:361–70.
11. McMahon JA, Green TJ, Skeaff CM, Knight RG, Mann JI, Williams SM. A controlled trial of homocysteine lowering and cognitive performance. N Engl J Med. 2006;354:2764–72.
12. Aisen PS, Schneider LS, Sano M, az-Arrastia R, van Dyck CH, Weiner MF, Bottiglieri T, Jin S, Stokes KT, et al. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. JAMA. 2008;300:1774–83.
13. Brady CB, Gaziano JM, Cxypoliski RA, Guarino PD, Kaufman JS, Warren SR, Hartigan P, Goldfarb DS, Jamison RL. Homocysteine lowering and cognition in CKD: The Veterans Affairs Homocysteine Study. Am J Kidney Dis. 2009;54:440–9.
14. Durga J, van Boxtel MPJ, Schouten EG, Kok FJ, Jolles J, Katan MB, Verhoef P. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. Lancet. 2007;369:208–16.
15. de Jager CA, Oulhaj A, Jacoby R, Refsum H, Smith AD. Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial. Int J Geriatr Psychiatry. 2012;27:.
16. Matthews RG, Sheppard C, Goulding C. Methylenetetrahydrofolate reductase and methionine synthase: biochemistry and molecular biology. Eur J Pediatr. 1998;157 Suppl 2:S54–9.
17. Brattström L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation. 1998;98:2520–6.
18. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111–3.
19. de Bree A, Verschuren WM, Bjorke-Monsen AL, van der Put NM, Heil SG, Trijbels FJ, Blom HJ. Effect of the methylenetetrahydrofolate reductase 677C→T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample. Am J Clin Nutr. 2003;77:687–93.
20. de Lau LM, van Meurs JB, Uitterlinden AG, Smith AD, Refsum H, Johnston C, Breteler MM. Genetic variation in homocysteine metabolism, cognition, and white matter lesions. Neurobiol Aging. 2010;31: 2020–2.
21. Bathum L, von Bornemann Hjelmborg J, Christiansen L, McGue M, Jeune B, Christensen K. Methylenetetrahydrofolate reductase 677C>T and methionine synthase 2756A>G mutations: no impact on survival, cognitive functioning, or cognitive decline in nonagenarians. J Gerontol A Biol Sci Med Sci. 2007;62:196–201.
22. Gussekloo J, Heijmans BT, Slagboom PE, Lagaay AM, Knook DL, Westendorp RG. Thermolabile methylenetetrahydrofolate reductase gene and the risk of cognitive impairment in those over 85. J Neurol Neurosurg Psychiatry. 1999;67:535–8.
23. Visscher PM, Tynan M, Whiteman MC, Pattie A, White I, Hayward C, Wright AF, Starr JM, Whalley LJ, Deary IJ. Lack of association between polymorphisms in angiotensin-converting-enzyme and methylenetetrahydrofolate reductase genes and normal cognitive ageing in humans. Neurosci Lett. 2003;347:175–8.
24. Roffman JL, Weiss AP, Deckersbach T, Freudenreich O, Henderson DC, Purcell S, Wong DH, Halsted CH, Goff DC. Effects of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism on executive function in schizophrenia. Schizophr Res. 2007;92:181–8.
25. Yoo JH, Choi GD, Kang SS. Pathogenicity of thermolabile methylenetetrahydrofolate reductase for vascular dementia. Arterioscler Thromb Vasc Biol. 2000;20:1921–5.
26. Bottiglieri T, Parnetti L, Arning E, Ortiz T, Amici S, Lanari A, Gallai V. Plasma total homocysteine levels and the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene: a study in an Italian population with dementia. Mech Ageing Dev. 2001;122: 2013–23.
27. Elkins JS, Johnston SC, Ziv E, Kado D, Cauley JA, Yaffe K. Methylenetetrahydrofolate reductase C677T polymorphism and cognitive function in older women. Am J Epidemiol. 2007;166:672–8.
28. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B-12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. Arch Gen Psychiatry. 2003;60:618–26.
29. Chen CS, Tsai JC, Tsang HY, Kuo YT, Lin HF, Chiang IC, Devanand DP. Homocysteine levels, MTHFR C677T genotype, and MRI hyperintensities in late-onset major depressive disorder. Am J Geriatr Psychiatry. 2005;13:869–75.
30. Gaysina D, Cohen S, Craddock N, Farmer A, Hoda F, Korszun A, Owen MJ, Craig IW, McGuffin P. No association with the 5,10-methylenetetrahydrofolate reductase gene and major depressive disorder: results of the depression case control (DeCC) study and a meta-analysis. Am J Med Genet B Neuropsychiatr Genet. 2008;147B:699–706.
31. Lewis SJ, Lawlor DA, Davey Smith G, Araya R, Timpson N, Day INM, Ebrahim S. The thermolabile variant of MTHFR is associated with depression in the British Women's Heart and Health Study and a meta-analysis. Mol Psychiatry. 2006;11:352–60.
32. Durga J, van Boxtel MP, Schouten EG, Bots ML, Kok FJ, Verhoef P. Folate and the methylenetetrahydrofolate reductase 677C→T mutation correlate with cognitive performance. Neurobiol Aging. 2006;27: 334–43.
33. de Lau LML, Refsum H, Smith AD, Johnston C, Breteler MMB. Plasma folate concentration and cognitive performance: Rotterdam Scan Study. Am J Clin Nutr. 2007;86:728–34.
34. Tucker KL. Stress and nutrition in relation to excess development of chronic disease in Puerto Rican adults living in the Northeastern USA. J Med Invest. 2005;52 Suppl:252–8.
35. Scott TM, Peter I, Tucker KL, Arsenault L, Bergethon P, Bhadelia R, Buell J, Collins L, Dashe JF, Griffith J, et al. The Nutrition, Aging, and Memory in Elders (NAME) study: design and methods for a study of micronutrients and cognitive function in a homebound elderly population. Int J Geriatr Psychiatry. 2006;21:519–28.
36. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal. 1999;14:143–9.
37. Sun X, Chiu CC, Liebson E, Crivello NA, Wang L, Claunch J, Folstein M, Rosenberg I, Mwamburi DM, Peter I, et al. Depression and plasma amyloid beta peptides in the elderly with and without the apolipoprotein E4 allele. Alzheimer Dis Assoc Disord. 2009;23:238–44.
38. Folstein MF, Robins LN, Helzer JE. The Mini-Mental State Examination. Arch Gen Psychiatry. 1983;40:812.
39. Radloff LS. The CES-D Scale: a self-report depression scale for research in the general population. Appl Psychol Measur. 1977;1:385–401.
40. Everitt BS, Landau S. The use of multivariate statistical methods in psychiatry. Stat Methods Med Res. 1998;7:253–77.
41. von Knorring L, Lindstrom E. Principal components and further possibilities with the PANSS. Acta Psychiatr Scand Suppl. 1995;388: 5–10.
42. Henderson M, Furnham A. Dimensions of assertiveness: factor analysis of five assertion inventories. J Behav Ther Exp Psychiatry. 1983;14:223–31.
43. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem. 2007;53:766–72.
44. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr. 1987;422:43–52.
45. Soldin SJ, Wan BS, Cherian AG. The measurement of creatinine: a comparison between the Beckman Creatinine Analyzer II and the Selective Analyzer GSA IID. Clin Biochem. 1981;14:165–8.
46. Shin YS, Rasshofer R, Friedrich B, Endres W. Pyridoxal-5′-phosphate determination by a sensitive micromethod in human blood, urine and tissues; its relation to cystathioninuria in neuroblastoma and biliary atresia. Clin Chim Acta. 1983;127:77–85.
47. Austin PC, Escobar M, Kopec JA. The use of the Tobit model for analyzing measures of health status. Qual Life Res. 2000;9:901–10.

48. Wang B, Jin F, Kan R, Ji S, Zhang C, Lu Z, Zheng C, Yang Z, Wang L. Association of MTHFR gene polymorphism C677T with susceptibility to late-onset Alzheimer's disease. J Mol Neurosci. 2005;27:23–7.
49. Bi XH, Zhao HL, Zhang ZX, Zhang JW. Association of RFC1 A80G and MTHFR C677T polymorphisms with Alzheimer's disease. Neurobiol Aging. 2009;30:1601–7.
50. Ravaglia G, Forti P, Maioli F, Scali RC, Arnone G, Talerico T, Pantieri T, Nativio V, Mantovani V, Bianchin M. Common polymorphisms in methylenetetrahydrofolate reductase (MTHFR): relationships with plasma homocysteine concentrations and cognitive status in elderly northern Italian subjects. Arch Gerontol Geriatr Suppl. 2004:339–48.
51. Almeida OP, Flicker L, Lautenschlager NT, Leedman P, Vasikaran S, van Bockxmeer FM. Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life. Neurobiol Aging. 2005;26:251–7.
52. Ramos MI, Allen LH, Haan MN, Green R, Miller JW. Plasma folate concentrations are associated with depressive symptoms in elderly Latina women despite folic acid fortification. Am J Clin Nutr. 2004;80:1024–8.
53. Ramos MI, Allen LH, Mungas DM, Jagust WJ, Haan MN, Green R, Miller JW. Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. Am J Clin Nutr. 2005;82:1346–52.
54. Elias MF, Robbins MA, Budge MM, Elias PK, Dore GA, Brennan SL, Johnston C, Nagy Z. Homocysteine and cognitive performance: modification by the *ApoE* genotype. Neurosci Lett. 2008;430:64–9.
55. Elias MF, Sullivan LM, D'Agostino RB, Elias PK, Jacques PF, Selhub J, Seshadri S, Au R, Beiser A, Wolf PA. Homocysteine and cognitive performance in the Framingham offspring study: age is important. Am J Epidemiol. 2005;162:644–53.
56. Kado DM, Karlamangla AS, Huang MH, Troen A, Rowe JW, Selhub J, Seeman TE. Homocysteine versus the vitamins folate, B-6, and B-12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. Am J Med. 2005;118:161–7.
57. Riggs KM, Spiro A III, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr. 1996;63:306–14.
58. Perna AF, Ingrosso D, Satta E, Lombardi C, Acanfora F, De Santo NG. Homocysteine metabolism in renal failure. Curr Opin Clin Nutr Metab Care. 2004;7:53–7.
59. Kurella Tamura M, Wadley V, Yaffe K, McClure LA, Howard G, Go R, Allman RM, Warnock DG, McClellan W. Kidney function and cognitive impairment in US adults: the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study. Am J Kidney Dis. 2008;52:227–34.
60. Need AC, Attix DK, McEvoy JM, Cirulli ET, Linney KL, Hunt P, Ge D, Heinzen EL, Maia JM, Shianna KV, et al. A genome-wide study of common SNPs and CNVs in cognitive performance in the CANTAB. Hum Mol Genet. 2009;18:4650–61.
61. Seshadri S, DeStefano AL, Au R, Massaro JM, Beiser AS, Kelly-Hayes M, Kase CS, D'Agostino RB Sr, DeCarli C, Atwood LD, et al. Genetic correlates of brain aging on MRI and cognitive test measures: a genome-wide association and linkage analysis in the Framingham Study. BMC Med Genet. 2007;8 Suppl 1:S15.