Associations Between Elevated Homocysteine, Cognitive Impairment, and Reduced White Matter Volume in Healthy Old Adults

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Objectives: *Elevated homocysteine has emerged as a risk factor for cognitive impairment even in healthy elderly persons. Reduced brain volume and white matter hyperintensities also occur in healthy elderly as well, but the interrelationships between these have not been well studied. We report these interrelationships in non demented, relatively healthy, community-dwelling older adults from a single East Asian population.* **Methods:** *Two hundred twenty-eight right-handed participants age 55 years and above were evaluated. Persons with medical conditions or neurological diseases other than well-controlled diabetes mellitus and hypertension were excluded. Participants underwent quantitative magnetic resonance imaging of the brain using a standardized protocol and neuropsychological evaluation. Plasma homocysteine, folate, vitamin B_{12}, and markers for cardiovascular risk: blood pressure, body mass index, fasting blood glucose, and lipid profile were measured.* **Results:** *Elevated homocysteine was associated with reduced global cerebral volume, larger ventricles, reduced cerebral white matter volume, and lower cognitive performance in several domains. Elevated homocysteine was associated with reduced white matter volume ($\beta = -20.80$, $t = -2.9$, $df = 223$, $p = 0.004$) and lower speed of processing ($\beta = -0.38$, $t = -2.1$, $df = 223$, $p = 0.03$), even after controlling for age, gender, and education. However, the association between homocysteine and lower speed of processing disappeared after controlling for white matter volume. Elevated homocysteine was not associated with white matter hyperintensity volume or with hippocampal volume. Although homocysteine and folate levels were correlated, their effects on white matter volume were dissociated.* **Conclusion:** *In non demented, relatively healthy adults, elevated homocysteine is associated with lower cognitive scores and reduced cerebral white matter volume. These effects can be dissociated from those related to white matter hyperintensities or reduced folate level.* (AM J Geriatr Psychiatry 2011; 00:1–9)

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Elevated plasma homocysteine is associated with impaired cognitive performance and increased risk for cognitive decline and of developing dementia,[1–7] in non demented older persons. Elevated homocysteine has been associated with a number of structural brain changes including global cerebral atrophy,[8] hippocampal atrophy,[9] silent brain infarction,[8,10] and increased white matter hyperintensities (WMH),[10–12] although negative findings have also been reported.[13,14]

How these structural changes might be related to homocysteine or how they could be the mediating factors to altered cognitive performance is presently unclear. For example, we do not know the relative extent to which gray or white matter volume loss contributes to cerebral atrophy, or, if such atrophy contributes to cognitive decline independent of increased WMH.[8,10]

To clarify these gaps in our knowledge, this cross-sectional study evaluated the interrelationships between brain structural changes, homocysteine levels, and cognitive performance. To reduce sources of variation that could contribute to varied structural imaging findings across different studies, we examined an ethnically homogenous population of healthy East Asians who were carefully screened for medical conditions. We concurrently collected detailed neuropsychological data and sampled blood for factors known to influence vascular risk and cognitive decline and controlled for various factors known to influence cognition.

METHODS

Participants

A total of 240 healthy, community-based volunteers who were part of the Singapore-Longitudinal Aging Brain Study participated in the study.[15] Participants were right-handed, of Han Chinese ethnicity, and age 55 years and above, with no known active medical condition other than uncomplicated and treated diabetes mellitus or hypertension.

Participants were excluded if they had any of the following: i) history of significant vascular events (i.e., myocardial infarction, stroke, or peripheral vascular disease); ii) history of malignant neoplasia of any form; iii) a history of cardiac, lung, liver, or renal failure; iv) active or an inadequately treated thyroid disease; v) active neurological or psychiatric conditions; vi) a history of head trauma with loss of consciousness; vii) a Mini-Mental State Examination[16] score less than 26; vii) a 15-point modified-Geriatric Depression Screening Scale[17] score greater than 9.

Participants could be excluded on the basis of disqualifying information obtained during the structured interview, results of blood tests, or self-reports of medication and supplement intake. The study was approved by the National University of Singapore institutional review board. All participants provided written informed consent prior to undergoing evaluation.

Laboratory Tests and Interview

Several blood-based markers of cardiovascular risk were studied and have been reported in brief previously.[15] Venous blood samples were drawn between 8:30 A.M. and 9:30 A.M. after an overnight fast and were tested for fasting blood glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, homocysteine, folate, creatinine, vitamin B_{12}, and apolipoprotein E (ApoE) genotype. As renal dysfunction can elevate homocysteine, persons with elevated creatinine were excluded. Plasma total homocysteine was measured using the automated chemiluminescent enzyme immunoassay method (Diagnostic Products Corporation, Los Angeles, CA); the coefficient of variation of the measurement ranged from 4.1% to 10.4%. Folate and vitamin B_{12} were measured in serum by radioassay with an Elecsys Folate II reagent kit (CVs ranged from 6.1% to 13.8%) and an Elecsys Vitamin B_{12} reagent kit (CVs ranged from: 3.2% to 7.6%; Roche Diagnostic, Indianapolis, IN), respectively. ApoE genotyping was identified by polymerase chain reaction amplification followed by

restriction endonuclease digestion of the polymerase chain reaction product (polymerase chain reaction–restriction fragment length polymorphism). A structured interview was conducted to collect data on participant social demographics, cigarette smoking, alcohol consumption, and medical history. Blood pressure and anthropometric measures were also taken.

Magnetic Resonance Imaging

Participants underwent magnetic resonance imaging (MRI) on a 3T Siemens Allegra system (Siemens, Erlangen, Germany) using a strictly protocol-driven imaging procedure that incorporated a number of quality control measures described in detail previously.[15] T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo images were acquired for morphometric analysis with a protocol similar to that used by the Alzheimer Disease Neuroimaging Initiative consortium,[18] TR = 2300 ms, TI = 900 ms, flip angle = 9°, BW: 240 Hz/pixel, FOV: 256 × 240 mm, 256 × 256 matrix; resulting voxel dimensions: 1.0 × 1.0 × 1.1 mm, acquisition time 9 minutes and 14 seconds.

We used a four-channel head coil and paid careful attention to head positioning within the coil. Parallel imaging was used to improve the signal-noise ratio. Images were inspected for motion artifact at the time of acquisition and scanning was repeated if necessary. The images were corrected for nonuniformity.[19] Three-dimensional–gradient unwarping was implemented[20] to reduce geometric distortions arising from gradient nonlinearity. Two-dimensional–fluid attenuated inversion recovery images obtained in the axial plane (TR = 10000 ms, TI = 2500 ms, TE = 96 ms, voxel dimensions 0.9 × 0.9 × 5.0 mm) were used to measure the volume of WMH. A neurologist reviewed the images for pathologicfeatures.

Image Analysis

The data were processed using a uniform MRI data-processing pipeline. Both manual and automated measurements were made. Two trained researchers performed manual, interactive volumetry for total intracranial volume, hippocampal volume (HC), ventricle volume, and WMH using Analyze 7.0 software (Mayo Clinic, Rochester, MN) on graphic tablets (Wacom DTU-710, Wacom Saitama, Japan). Details concerning the measurement technique and the landmarks used for manual volumetry have been described previously.[15] Inter-tracer reliability for manually traced volumes was evaluated by comparing measurements of 10 randomly-selected brains made by two tracers on two different occasions that were separated by at least 4 weeks. The intra-class correlation coefficients were 0.93 for HC, 0.99 for total intracranial volume, 0.99 for WMH, and 0.99 for ventricle measurements.

Automated measurement was performed using FreeSurfer 3.0.5 software (http://surfer.nmr.mgh.harvard.edu/; Martinos Imaging Centre, Charlestown, MA). Morphometric evaluation of each brain hemisphere was conducted independently. Although FreeSurfer generates many measurements of brain structure, we only report the results for total cerebral volume (TCV), gray matter, and white matter volumes. All brain variables were corrected for head size using an analysis of covariance approach prior to statistical analysis.[21]

Cognitive Testing

Participants were assessed at a fixed time of day, between 10 A.M. and 2 P.M. and within 3 months of undergoing MRI. A battery of 11 neuropsychological tests evaluating six cognitive domains—attention, verbal memory, nonverbal memory, executive functioning, speed of processing, and language was used. We minimized the effects of language and culture by using tests that contained items that were relatively familiar to the study population. Attention was assessed using the Digit Span subtest from the Wechsler Memory Scale III[22] and a computerized version of a Spatial Span task. Verbal memory was evaluated using the Rey Auditory Verbal Learning Test[23] and a Verbal-Paired Associates test. Visuospatial memory was evaluated using the visual reproduction subtest from the Wechsler Memory Scale III and a Visual Paired Associates test. Executive functioning was assessed using a Categorical Verbal Fluency test (using categories of animals, vegetables and fruits), the Design Fluency test,[24] and the Trail Making Test B.[25] Speed of processing was assessed with the Trail-Making Test A[25] and the Symbol-Digits Modalities Test.[26] Language was evaluated using the

Object and Action Naming Battery.[27] The tests were administered in either English or Mandarin according to the subject's most proficient language. The individual test scores were standardized (z-transformed) and combined into six theoretically motivated composite scores (attention, verbal memory, visuospatial memory, speed of processing, executive functions, and language) to limit the number of comparisons.

Statistical Analysis

Data analysis was conducted on 228 participants, after excluding 8 participants who had missing homocysteine results, and 12 participants who had unsuitable structural brain imaging data. Distribution of homocysteine, folate and vitamin B_{12} were significantly skewed, and thus were subjected to natural log-transformation to improve normality.

Multiple linear regression was used to examine the relationships between age, cognitive performance, and brain structure, as well as between log-transformed plasma total homocysteine and MRI volumetric measures. We controlled for the effects of potential confounding variables while assuming a linear relationship between homocysteine and brain measures. In these analyses, age, gender, and education were covariates, as a prior study[15] showed that they influence cognitive scores and possibly brain volumes. Additional adjustments for body mass index, systolic blood pressure, high-density lipoprotein cholesterol, creatinine, folate, vitamin B_{12} ApoE4 genotype, smoking, alcohol intake, and white matter hyperintensity volume were performed by entering the variables to the model sequentially.

In addition to the above, we examined whether brain volumetric measures contributed independently to the relationship between homocysteine and cognitive performance. Composite cognitive scores were examined as dependent variables in hierarchical models. Age, gender, years of education, and log-transformed homocysteine values were first entered into the model as independent variables. MRI measures were then added. We used the Sobel test to test whether cerebral white matter volume mediates homocysteine-related changes in cognitive performance. The Sobel test determines if the effect of the mediator on the dependent variable is significantly different from zero using a 2-tailed z test with ±1.96 as the critical value in a unit normal distribution.[28,29] Statistical analyses were conducted using SPSS 18.0 software (IBM SPSS Inc., Chicago, IL).

RESULTS

The mean age of the study participants was 65.4 years (SD: 6.2 years), and a slightly greater proportion were women (53.9%) (Table 1). The average number of years of education was 10.7 (SD: 3.4 years). There were relatively few current smokers (3.1%) and few regular alcohol drinkers (11.9%). We took an all-inclusive approach to reporting hypertension and diabetes, counting *any* individual who either self-reported these conditions or had any history of having been administered medication for these conditions. This led to the relatively high proportions of hypertension (41.7%) and diabetes (12.7%) (Table 1). Against this, it should be noted that blood pressure and fasting blood glucose measures obtained correspond to those derived from a healthy population. Mini-Mental State Examination score averaged 28.5 (SD: 1.2, range: 26–30).

The mean value of total plasma homocysteine was 13.4 μmol/L (SD: 3.8, range: 6.3–27.1) and 31.1% of the participants had homocysteine levels above 15 μmol/L, the upper limit of the laboratory reference range for this population. Increasing age was correlated with higher homocysteine ($r = 0.22$, $df = 226$ $p = 0.001$) and men were more likely to have higher homocysteine levels than women ($t = 7.6$, $df = 226$, $p < 0.001$). Log-transformed homocysteine level correlated with higher systolic blood pressure ($r = 0.15$, $df = 226$, $p < 0.03$), higher body mass index ($r = 0.22$, $df = 226$, $p = 0.001$), lower high-density lipoprotein cholesterol level ($r = -0.27$, $df = 226$, $p < 0.001$), lower serum folate level ($r = -0.47$, $df = 226$, $p < 0.001$) and lower vitamin-B12 level ($r = -0.40$, $df = 226$, $p < 0.001$). Homocysteine level did not correlate with diastolic blood pressure, fasting glucose level, or calculated low-density lipoprotein cholesterol levels.

Age, Brain Structure, and Cognitive Performance

Increasing age was associated with reduced TCV (TCV; $\beta = -3.78$, $t = -7.8$, $df = 226$, $p < 0.001$), HC ($\beta = -0.03$, $t = -4.8$, $df = 226$, $p < 0.001$), cerebral white matter volume ($\beta = -1.85$, $t = -6.6$, $df = 226$, $p < 0.001$)

TABLE 1. Characteristics of the Study Participants (N = 228)

Variable	Value
Age in years, mean (SD)	65.4 (6.2)
Age > 65 years, n (%)	107 (46.9)
Female, n (%)	123 (53.9)
Number of years of education, mean (SD)	10.7 (3.4)
Current smoker, n (%)	7 (3.1)
Regular alcohol drinker (≥1 drink/week), n (%)	27 (11.9)
Systolic BP (mm Hg), mean (SD)	131.4 (15.8)
Diastolic BP (mm Hg), mean (SD)	80.2 (8.9)
Creatinine (mmol/L), mean (SD)	83.3 (19.7)
Fasting blood glucose (mmol/L), mean (SD)	5.2 (1.0)
LDL-C (mmol/L), mean (SD)	3.3 (0.7)
HDL-C (mmol/L), mean (SD)	1.5 (0.4)
Folate (nmol/L), mean (SD)	25.9 (16.0)
Vitamin B-12 (pmol/L), mean (SD)	427.5 (217.3)
Hypertension, n (%)	95 (41.7)
Diabetes mellitus, n (%)	29 (12.7)
BMI, mean (SD)	23.4 (3.0)
ApoE-ε4 heterozygotes, n (%)	44 (19.4)
Homocysteine (μmol/L), mean (SD)	13.4 (3.8)
Hyperhomocysteinemia,[a] n (%)	71 (31.1)
MMSE total score, mean (SD)	28.5 (1.2)
MRI volumetric measures[b]	
TCV (cm^3), mean (SD)	873.4 (50.9)
Hippocampus (cm^3), mean (SD)	6.5 (0.6)
Ventricular volume,[c] mean (SD)	1.3 (0.2)
WMH volume[c] (cm^3), mean (SD)	2.0 (0.7)
Cerebral white matter volume (cm^3), mean (SD)	437.3 (28.5)
Cerebral gray matter volume (cm^3), mean (SD)	397.3 (20.2)
Standardized cognitive composite scores	
Attention, mean (SD)	−0.05 (2.5)
Verbal memory, mean (SD)	−0.16 (6.4)
Nonverbal memory, mean (SD)	0.02 (4.9)
Language, mean (SD)	−0.01 (1.9)
Speed of processing, mean (SD)	0.01 (2.5)
Executive function, mean (SD)	0.03 (4.3)

Notes: ApoE-ε4: apolipoprotein E; BMI: body mass index; BP: blood pressure; eTIV: estimated total intracranial volume from FreeSurfer; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MMSE: Mini-Mental State Exam; MRI: magnetic resonance imaging; TCV: total cerebral volume; TIV: total intracranial volume; WMH: white matter hyperintensities.

[a]Defined as plasma total homocysteine greater than 15 μmol/L.

[b]All volumes were adjusted for total intracranial volume (TIV or eTIV as appropriate).

[c]Ventricular and total white matter intensities volumes were log-transformed.

and cerebral gray matter volume ($\beta = -1.07$, $t = -5.2$, $df = 226$, $p < 0.001$). There were significant age-related increases in log-transformed ventricular volume ($\beta = 0.012$, t: 6.8, df: 226, $p < 0.001$) and log-transformed WMH ($\beta = 0.044$, $t = 6.0$, $df = 226$, $p < 0.001$). Gender and education did not significantly influence the effect of age on these measures of brain structure.

Increasing age was also associated with lower cognitive performance. The detrimental effects of age on attention ($\beta = -0.021$, $t = -3.2$, $df = 226$, $p = 0.001$), verbal memory ($\beta = -0.020$, $t = -2.7$, $df = 226$, $p = 0.006$), nonverbal memory ($\beta = -0.026$, $t = -3.6$, $df = 224$, $p < 0.001$), speed of processing ($\beta = -0.057$, $t = -7.1$, $df = 226$, $p < 0.001$), executive function ($\beta = -0.028$, $t = -4.3$, $df = 226$, $p < 0.001$) and language ($\beta = -0.033$, $t = -3.4$, $df = 226$, $p = 0.001$) were all significant and remained significant after controlling for gender and education.

Homocysteine and Brain Structure

Elevated log-transformed homocysteine level was associated with lower TCV ($\beta = -24.72$, $t = -2.1$, $df = 226$, $p = 0.04$) and higher ventricular volume ($\beta = 0.12$, $t = 3.0$, $df = 226$, $p = 0.003$). Homocysteine level did not correlate with HC ($\beta = -0.03$, $t = 1.7$, $df = 226$, $p = 0.84$). Elevated homocysteine level was associated with reduced cerebral white matter volume ($\beta = -28.18$, $t = -4.3$, $df = 226$, $p < 0.001$) but not reduced gray matter volume ($\beta = -8.35$, $t = -1.7$, $df = 226$, $p = 0.08$). The association between elevated homocysteine level and reduced cerebral white matter volume remained significant after controlling for the effects of age, gender, and education ($\beta = -20.80$, $t = -2.9$, $df = 223$, $p = 0.004$), whereas its association with reduced TCV and higher ventricular volume did not remain significant (Table 2, Model 2).

The association between homocysteine and reduced white matter volume remained significant even after further adjustment for systolic blood pressure, body mass index, high-density lipoprotein, creatinine, fasting glucose, ApoE4 genotype, folate, vitamin B_{12}, smoking, and alcohol intake: the adjusted regression coefficient was −23.8, ($t = -2.6$, $df = 208$, $p = 0.01$).

Homocysteine was not associated with volume of WMH ($\beta = 0.13$, $t = 0.7$, $df = 226$, $p = 0.44$). Adjustment for WMH did not affect the independent effect of homocysteine on cerebral white matter volume ($\beta = -24.48$, $t = -2.6$, $df = 208$, $p = 0.009$). Although correlated with homocysteine levels,[30] folate and vitamin B_{12} levels did not show significant correlation with structural brain measures (all $p > 0.10$; Table 3).

Homocysteine and Cognitive Performance

Elevated homocysteine level was associated with reduced performance in multiple cognitive domains

TABLE 2. Association Between Log-Transformed Homocysteine on Brain MRI Volumetric Measures

Dependent Variable	Model 1[a]				Model 2[b]				Model 3[c]			
	β[d]	SE	*t* (*df*)	p	β	SE	*t* (*df*)	p	β	SE	*t* (*df*)	p
TCV	−24.7	11.9	−2.1 (226)	0.04	−8.86	12.7	−0.6 (223)	0.48	−18.2	16.4	0.9 (208)	0.30
Total HC	−0.03	0.02	1.7 (226)	0.84	0.19	0.16	1.2 (223)	0.24	0.26	0.21	1.3 (208)	0.12
Ventricular volume[e]	0.12	0.04	3.0 (226)	0.003	0.06	0.04	1.3 (223)	0.18	0.03	0.05	0.4 (208)	0.64
WMH[e]	0.13	0.17	0.7 (226)	0.44	−0.01	0.19	0.01 (223)	0.96	−0.09	0.24	−0.1 (208)	0.73
Cerebral white matter volume	−28.2	6.49	−4.3 (226)	<0.001	−20.8	7.17	−2.9 (223)	0.004	−23.8	9.24	−2.6 (208)	0.01
Cerebral gray matter volume	−8.35	4.78	−1.7 (226)	0.08	0.53	5.36	0.1 (223)	0.92	3.85	6.82	0.7 (208)	0.60

Notes: A p value <0.05 indicates statistical significance. HC: hippocampal volume; MRI: magnetic resonance imaging; TCV: total cerebral volume; WMH: white matter hyperintensities.
[a]Unadjusted association between homocysteine and brain measures.
[b]Adjustment was made for age, gender, and education in years.
[c]In addition, adjusted for systolic blood pressure, body mass index, high-density lipoprotein, creatinine, fasting glucose, ApoE-ε4 genotype, folate, vitamin B_{12}, smoking, and alcohol intake.
[d]Regression coefficients, standard error (SE), *t* test, degree of freedom (*df*), and p values were computed using multiple linear regression.
[e]Ventricular volumes and total white matter hyperintensities volumes were log-transformed.

TABLE 3. Association Between Log-Transformed Folate and Log-Transformed Vitamin B_{12} on Brain MRI Volumetric Measures

Dependent Variable	Log-Transformed Folate				Log-Transformed Vitamin B-12			
	β[a]	SE	*t* (*df*)	p	β[a]	SE	*t* (*df*)	p
TCV (cm^3)	−0.96	5.75	−0.2 (223)	0.86	−6.67	7.48	−0.9 (226)	0.37
Total HC (cm^3)	−0.01	0.07	−0.2 (223)	0.83	−0.07	0.09	−0.7 (226)	0.46
Ventricular volume[b]	−0.02	0.02	−0.8 (223)	0.40	−0.002	0.03	−0.1 (226)	0.93
WMH[b]	0.06	0.08	0.6 (223)	0.50	−0.02	0.10	−0.2 (226)	0.86
Cerebral white matter volume (cm^3)	3.61	3.17	1.1 (223)	0.29	−2.39	4.18	−0.5 (226)	0.56
Cerebral gray matter volume (cm^3)	1.31	2.27	0.6 (223)	0.56	−3.93	2.97	−1.3 (226)	0.18

Notes: A p value <0.05 indicates statistical significance. HC, hippocampal volume; TCV, total cerebral volume; WMH, white matter hyperintensities.
[a]Regression coefficients, standard error (SE), *t* test, degree of freedom (*df*) and p values were computed using multiple linear regression.
[b]Ventricular volumes and total white matter hyperintensity volumes were log-transformed.

after adjusting for age: verbal memory ($\beta = -0.67$, $t = -4.2$, $df = 226$, p <0.001), nonverbal memory ($\beta = -0.47$, $t = -2.9$, $df = 224$, p <0.004), speed of processing ($\beta = -0.36$, $t = -2.1$, $df = 226$, p = 0.04), and executive function ($\beta = -0.36$, $t = -2.5$, $df = 226$, p = 0.01). An independent effect of elevated homocysteine level on speed of processing ($\beta = -0.38$, $t = -2.1$, $df = 223$ p = 0.03) was evident after controlling for age, gender, and education (Table 4, Model 2).

Concurrently cerebral white matter volume was associated with speed of processing ($\beta = 0.005$, $t = 2.9$, $df = 223$, p = 0.004) and executive function ($\beta = 0.004$, $t = 3.1$, $df = 223$, p = 0.002) after adjusting for age, gender, and education. The inclusion of cerebral white matter volume in the multiple regression analysis (Table 4, Model 3) attenuated the previously significant association between elevated homocysteine and reduced speed of processing.

In mediation analyses using Sobel tests, we found that homocysteine significantly mediated age-related reduction in cerebral white matter volume (Z = –2.22, p = 0.027). The volume of cerebral white matter significantly mediated homocysteine related deficits in speed of processing (Z = –2.94, p = 0.003).

TABLE 4. Log-Transformed Homocysteine and Cognitive Performance, With Hierarchical Adjustment for Cerebral White Matter Volume

	Model 1[a]				Model 2[b]				Model 3[c]			
Variables	**β[d]**	**SE**	***t* (*df*)**	**p**	**β[d]**	**SE**	***t* (*df*)**	**p**	**β[d]**	**SE**	***t* (*df*)**	**p**
Attention	0.003	0.14	0.02 (226)	0.98	−0.14	0.16	−0.8 (223)	0.37	−0.09	0.16	−0.4 (222)	0.58
Verbal memory	−0.67	0.16	−4.2 (226)	<0.001	−0.28	0.17	−1.6 (223)	0.09	−0.23	0.17	−1.3 (222)	0.18
Nonverbal memory	−0.47	0.16	−2.9 (224)	0.004	−0.30	0.18	−1.6 (221)	0.09	−0.26	0.18	−1.4 (220)	0.15
Speed of processing	−0.36	0.18	−2.1 (226)	0.04	−0.38	0.17	−2.1 (223)	0.03	−0.29	0.18	−1.6 (222)	0.11
Executive function	−0.36	0.14	−2.5 (226)	0.01	−0.28	0.15	−1.8 (223)	0.06	−0.20	0.15	−1.4 (222)	0.15
Language	−0.33	0.22	−1.5 (225)	0.13	−0.42	0.22	−1.8 (222)	0.06	−0.38	0.23	−1.6 (221)	0.09

Notes: A p value <0.05 indicates statistical significance. CI: confidence interval.
[a]Association between homocysteine and cognitive test scores, adjusted for age (years).
[b]Further adjustment was made for gender and years of education.
[c]In addition to the variables in model 2, further adjustment was made for cerebral white matter volume.
[d]Regression coefficients, standard error (SE), *t* test, degree of freedom (*df*), and p values were computed using multiple linear regression.

DISCUSSION

This ethnically homogenous cohort of relatively healthy elderly persons enabled us to highlight the interrelationships between elevated homocysteine, brain structure, and cognitive performance.

Homocysteine and Brain Structure

Elevated homocysteine is an established risk factor for adverse cerebrovascular outcomes[31] and brain infarction,[8,10] but it also has other effects on brain structure. The results concur with prior studies with respect to global cerebral atrophy but differ with respect to HC and increased white matter hyperintensity volume. The lack of association between homocysteine and white matter hyperintensity volume could be contributed by the relatively younger age of the present cohort, which was on average a decade younger than in many similar studies on healthy elderly reported from Caucasian dominant populations.[1,10] Both homocysteine level[32] and WMH[33] increase with age, and these factors could interact to contribute to cognitive decline. Such an interaction between age and structural change could also contribute to why we did not observer a correlation between lower HC and homocysteine level despite finding age-related HC loss.

In this study, the association between elevated homocysteine and cerebral atrophy[8] was driven by changes in white matter volume rather than gray matter volume. This contrasts with the balanced effect on gray and white matter volume observed with increasing age. Although age typically accounts for most of the variance in brain structure and cognitive performance,[15,34,35] the association between elevated homocysteine and reduced white matter volume remained significant even after controlling for age.

This independent association between homocysteine level and white matter volume was also not related to WMH volume. This underscores the point that different measures of white matter integrity may make dissociable contributions to reduced cognitive performance[36] despite being correlated.[37]

Low folate and vitamin B_{12} levels have been negatively correlated with homocysteine level[3,38] and our findings concur. However, despite their common links with one-carbon transfer reactions relevant to nervous system function, we found dissociation between the effects of folate and homocysteine on cerebral white matter volume. Folate and homocysteine may thus index different effects on the aging brain. Endothelial dysfunction,[39] increased oxidative stress[40,41] and increased rate of accumulation of amyloid[42,43] are potential mechanisms through which elevated homocysteine might exert negative effects on the brain, distinct from the effects of low folate.

Homocysteine levels can be lowered by folate supplementation. However, the results of intervention

have been mixed[44,45] and there remains concern that indiscriminate supplementation might elevate the risk of cancer[46] motivating the investigation of how homocysteine might mediate cognitive impairment.

Thepresent findings may contribute toward explaining why folate supplementation has shown mixed success in modulating cognition in persons with elevated homocysteine.[44,45] They may also encourage the search for mechanisms of cognitive impairment for which homocysteine is a valid marker but for which the link with folate is irrelevant.

Homocysteine and Cognitive Performance

The finding of a linear relationship between log-transformed elevated homocysteine levels and poorer performance over multiple cognitive domains agrees with earlier findings.[3–5] After controlling for age (as well as gender and education), the relationship between elevated homocysteine, reduced white matter volume, and speed of processing remained significant. Indeed, our findings concerning the effects of age on brain structure and cognitive function, suggest interactions involving homocysteine levels, cognitive performance, brain structure, and age, that merit confirmation in a longitudinal study. As the association between elevated homocysteine and reduced speed of processing disappeared when the analysis was adjusted for white matter volume, it seems reasonable to suggest that elevated homocysteine could mediate lower cognitve performance via reduction of cerebral white matter volume.

In sum, we propose several points regarding the interrelationships between elevated homocysteine, brain structure, and cognitive performance. In particular, we observed that in this ethnically homogenous cohort, changes in speed of processing are related to elevated homocysteine level and reduction in cerebral white matter volume. These associations with homocysteine seem to be dissociated from those related to WMH or reduced folate levels.

Extending the current cross-sectional findings to longitudinal data collection would additionally clarify age-by-homocysteine interactions and could further inform concerning how different structural changes correspond to cognitive decline in elderly persons. It would also be interesting to evaluate how these findings might relate to differences between East Asians and Westerners.

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