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Homocysteine as a Risk Factor for Cognitive Impairment in Stroke Patients

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Key Words
Brain scans · Cognitive impairment · Homocysteine · MRI · Total homocysteine

Abstract
Background: Elevated total homocysteine (tHcy) levels are associated with an increased risk of cerebrovascular disease. It is uncertain whether tHcy is also an independent risk factor for cognitive impairment. ***Methods:*** We examined 95 stroke subjects 3 months after their strokes, and 55 healthy comparison subjects, with a detailed neuropsychological assessment, and MRI brain scans in a proportion (n = 97). Baseline measurements of tHcy, serum folate and B_{12}, creatinine and plasma fibrinogen levels were obtained. ***Results:*** tHcy levels were higher in the stroke subjects by a mean 34%. These levels were significantly correlated with the first factor of a principal component analysis of the neuropsychological data, after controlling for age, folate, B_{12} and creatinine levels. The correlation of Hcy levels was particularly significant with frontal-executive functioning and attention. tHcy levels were significantly correlated with number of infarcts and total stroke volume in the stroke group, but not with T_2-weighted deep white matter hyperintensity scores, after correction for age. In the control group, tHcy levels were significantly correlated with ventricle-to-brain ratios as measures of brain atrophy. ***Conclusion:*** This study provides evidence that high tHcy levels are associated with cognitive impairment, in particular that of frontal-executive function. The major component of this association is accounted for by small and large strokes, but non-vascular neurotoxic effects of tHcy also appear to play a role. tHcy must receive greater attention as a risk factor for cognitive impairment.

Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of the essential amino acid methionine. Elevated plasma homocysteine levels were first linked with vascular disease by McCully [1]. Recent studies have confirmed hyperhomocysteinaemia (HHcy) to be associated with atherosclerotic disease in coronary, cerebral and peripheral blood vessels [2–4]. While severe HHcy is rare, mild elevation of blood Hcy levels has been reported to occur in 5–7% of the general population [5, 6], 30% of patients with coronary artery disease and 42%

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with cerebrovascular disease [7]. In the European Concerted Action Project [8], the magnitude of increased risk for vascular disease associated with HHcy (odds ratio, OR, for highest to lower quintiles = 2.2) was higher than that for hypercholesterolemia (OR 1.4), less than that for hypertension (OR 3.9), and similar to that for tobacco use (OR 2.2).

A high level of Hcy is both proatherogenic and prothrombotic [7]. Since HHcy is a risk factor for stroke [9, 10], and has been linked with higher rates of microangiopathy (i.e. vascular leucoencephalopathy and lacunar infarctions) [11], it is a risk factor for vascular dementia [12]. More recently, it has been argued that HHcy may produce cognitive impairment independently of stroke or other cerebrovascular disease. Riggs et al. [13] reported a relationship between Hcy levels and performance on certain cognitive tests in healthy individuals. Evers et al. [11] found patients with HHcy to have impairment in cognitive processing as measured by visual event-related potentials. Clarke et al. [14] demonstrated in 164 Alzheimer's disease (AD) subjects (76 with autopsy-confirmed AD) that Hcy levels were elevated and stable over time. In a study of memory clinic patients [15], Hcy was found to be an independent predictor of mini-mental state scores. A recent longitudinal study found plasma Hcy level measured 8 years earlier to be a strong, independent risk factor for the development of dementia and AD [16].

If Hcy promotes cognitive impairment and is a risk factor for AD, it has important epidemiological implications. In this study, we examined plasma Hcy levels in a stroke sample to determine if there was an association between Hcy levels and cognition, and if this could be accounted for by infarct size or small vessel disease as detected by MRI.

Methods

Sample

Subjects (n = 95) were consecutive patients admitted to two large teaching hospitals affiliated with the University of New South Wales who had recently suffered an ischemic stroke as diagnosed by two neurologists, and who met the diagnostic criteria for cerebral infarction [17]. Subjects were aged 55–85 years, did not have a diagnosis of dementia or other neurological disorder prior to the stroke, were not currently aphasic and were well enough to consent to participate. Healthy comparison subjects (n = 55) were age-matched, unpaid volunteers recruited from the neighbourhood community who had no history of stroke or other neurological or psychiatric disorder.

Assessment

Stroke subjects had a baseline assessment within 1 week of admission which included a detailed medical history and examination, history of risk factors for cerebrovascular disease and dementia, a functional assessment and the Mini-Mental State Examination [18]. Laboratory measurements included total Hcy (tHcy), serum B_{12} and folate levels, creatinine and plasma fibrinogen. Between 3 and 6 months after the stroke, a detailed neuropsychological assessment was performed and 97 subjects (55 stroke patients and 42 controls) had a brain MRI scan. The comparison group had a similar assessment performed in one stage.

Homocysteine Measurement

Fasting blood was collected and centrifuged within 6 h, and the plasma stored at –20°C for later analysis. Total Hcy was measured using a fluorescence-based immunochemical technique with demonstrated high repeatability [19, 20].

Neuropsychological Assessment

Premorbid ability was estimated on the basis of performance on the National Adult Reading Test [21]. Screening for depression was carried out as part of the psychiatric assessment, and the Geriatric Depression Scale [22] was administered. The battery comprised the following tests pertaining to various cognitive domains: handedness [23], attention (Wechsler Memory Scale-Revised, WMS-R, Mental Control Subtest, Wechsler Adult Intelligence Scale-Revised, WAIS-R, Digit Span subtest) [24]; speed of information processing (Trail Making Test Part A, Symbol Digit Modalities Test) [25]; verbal memory (Logical Memory I and II from WMS-R), visual memory (Visual Reproduction I and II from WMS -R) [26]; language (Modified Boston Naming Test) [27, 28], Modified Token Test [29], Sentence Repetition [30]; visuospatial and visuoconstructional ability – copying simple figures [31], clock drawing [30], WAIS-R Block Design Subtest [24]; arithmetical ability, WAIS-R Arithmetic Subtest [24]; gnosis (finger gnosis and stereognosis) [32]; praxis (Western Aphasia Battery praxis items) [33]; executive function, WAIS-R Similarities Subtest [24], Picture Completion Subtest (WAIS-R), Mattis Dementia Rating Scale Identities and Oddities Subtest [34], Colour Form Sorting Test [35], verbal generativity (FAS and animals) [36] and Trail Making Test Part B [37].

MRI Brain Scans

MRI was performed on a proportion of subjects (n = 55 stroke; n = 42 controls) using a 1.5-T Signa GE magnet and the following protocol: a scout mid-sagittal cut (2D, TR 300 ms, TE 14 ms; 5 mm thick, number of excitations 1.5); 1.5-mm-thick T_1-weighted contiguous coronal sections through whole brain using a FSPGR sequence and 3D acquisition (TR 14.3 ms, TE 5.4 ms); 4-mm-thick T_2-weighted FLAIR coronal slices through whole brain (TR 8900, TE 145, TI 2200, FOV 25, 256 × 192). Fifty-three subjects did not receive MRI scans because of claustrophobia or unwillingness to undergo the test.

Analysis of Data

Neuropsychological Tests. Raw scores were converted to age-scaled scores using published norms [24–26, 28, 36, 38–41]. Composite z-scores were obtained for each domain. The raw scores from individual tests were also used in the exploratory analyses.

MRI Scans. These were rated by a trained rater with good inter-rater (κ scores from 0.7 to 0.9 on various measures) and intra-rater (κ 0.8 to 0.9) reliability determined on five scans each. All ratings were carried out on a computer console using ANALYZE (Mayo Foundation) software. Brain infarctions were identified on T_1-

Table 1. Mean (SD) group values for demographic, serum, brain imaging and cognitive parameters

	Controls (n = 55)	Stroke patients (n = 95)	t value (d.f.)	p value
Demographics				
Age, years	71.7 (6.8)	72.7 (8.6)	–0.9 (148)	0.35
Males, %	50.6	56.6	$\chi^2 = 8.7$	<0.001
Education, years	11.7 (3.3)	10.1 (2.6)	3.9 (148)	<0.001
NART IQ[1]	111.9 (7.7)	105.3 (13.3)	4.0 (148)	<0.001
Blood testing				
tHcy, μmol/l	13.2 (4.5)	17.7 (7.2)	–4.2 (148)	<0.001
Creatinine, mmol/l	0.079 (0.02)	0.092 (0.03)	3.5 (148)	0.001
T_4, pmol/l	14.6 (3.1)	17.9 (10.9)	–2.5 (142)	0.006
TSH, mU/l	1.5 (1.3)	2.4 (2.9)	–2.70 (143)	0.007
B_{12}, pmol/l	324.7 (332.5)	271.8 (153.1)	1.3 (146)	0.188
Folate, nmol/l	20.2 (9.4)	19.4 (9.5)	0.5 (144)	0.639
Neuropsychological summary z-scores[4]				
Attention	1.4 (2.4)	–0.8 (3.5)	5.0 (148)	0.001
Executive	1.6 (2.3)	–1.0 (3.2)	6.4 (148)	0.001
Memory	0.9 (2.9)	–0.6 (3.4)	3.3 (148)	0.001
Language	1.0 (1.8)	–0.6 (2.3)	5.3 (148)	0.001
Brain imaging				
Total stroke volume, mm[3]	39.6	1,509.5	–3.2 (95)	0.002
VBR[2] anterior	0.32	0.33	–0.3 (95)	0.762
midsection	0.22	0.23	–0.6 (95)	0.387
Whole brain hyperintensity score[3]	5.6	7.6	–2.8 (95)	0.002
PVH score[3]	3.3	3.8	–2.0 (95)	0.066
DWMH score[3]	2.1	3.5	–3.2 (95)	<0.001
Internal capsule hyperintensity score[3]	1.9	3.2	–3.5 (95)	<0.001

[1] Intelligence quotient based on performance on the National Adult Reading Test [21].
[2] Ventricle to brain ratio (see text).
[3] Summary ratings of T_2-weighted hyperintensities (see text).
[4] Summary scores comprise a linear sum of whole group z-scores based on raw scaled scores calculated from indicative individual neuropsychological tests.

weighted images and each infarction delineated manually to obtain its area, which multiplied by thickness gave the total stroke volume. Periventricular (PVH) and deep white matter hyperintensities (DWMH) were rated on a 0–3 scale [42]. For PVH, ratings were performed for the lining of the lateral ventricles (rims) and the frontal and occipital horns (caps), the sum of which on either side gave the total PVH score (maximal score = 18). For DWMH rating, the frontal, temporo-parietal and occipital white matter, and the internal capsules were rated separately, and the scores for both sides added to give a total DWMH score (maximal score = 24). As a measure of atrophy, two ventricle-brain ratios (VBR, anterior and midsection) were obtained as described by Victoroff et al. [43].

Statistical Analysis. The two groups were compared on sociodemographic, neuropsychological, brain imaging and laboratory parameters. tHcy levels were correlated with neuropsychological and brain imaging parameters, as well as with folate, B_{12}, cholesterol, fibrinogen and creatinine levels. The relationship of tHcy with age and gender was examined. The neuropsychological data were subjected to a principal component analysis, and the first factor was correlated with tHcy levels, with corrections for age, folate and B_{12} levels and creatinine. Linear regression models were used to examine the independent effects of stroke and white matter hyperintensity characteristics, age and tHcy and to determine the direct and indirect effects of tHcy on cognition.

Results

Sample Characteristics

The characteristics of the sample are described in table 1. The two groups were closely matched on age, but there were significantly more men in the stroke sample

Table 2. Correlations between tHcy levels and cognitive performance

Correlate	Whole group (n = 145)	Controls (n = 52)	Stroke patients (n = 93)
Zero order			
Attention[1]	–0.26 (0.001)	–0.18 (0.19)	–0.16 (0.14)
Executive[1]	–0.29 (0.001)	–0.14 (0.33)	–0.2 (0.05)
Memory[1]	–0.26 (0.001)	–0.28 (0.04)	–0.18 (0.085)
Language[1]	–0.23 (0.006)	–0.21 (0.12)	–0.09 (0.407)
Age	0.25 (0.003)	0.40 (0.003)	0.2 (0.057)
Controlling for age, folate and creatinine			
Attention[1]	–0.24 (0.005)	–0.02 (0.912)	–0.13 (0.297)
Executive[1]	–0.24 (0.006)	0.06 (0.695)	–0.20 (0.104)
Memory[1]	–0.15 (0.083)	–0.14 (0.795)	–0.11 (0.376)
Language[1]	–0.05 (0.576)	–0.04 (0.795)	0.08 (0.532)
TMT-A[2]	0.23 (0.014)	0.01 (0.949)	0.20 (0.102)
TMT-B[3]	0.28 (0.003)	–0.13 (0.412)	0.30 (0.013)

Probability of zero order and partial correlations is given in parentheses.

[1] Summary scores on cognitive domains, which comprise a linear sum of whole group z-scores based on raw scaled scores calculated from indicative individual neuropsychological tests.

[2] Trails Making Test A.

[3] Trails Making Test B.

(51% in the control group, 57% in the stroke group; χ^2 = 8.7, $p < 0.01$). The control group had received, on an average, 1.6 years more of education ($t = 3.9$, $p < 0.001$) and had a higher IQ as measured on the National Adult Reading Test ($t = 4.0$, $p < 0.001$).

tHcy levels were significantly higher in the stroke sample by a mean 34% ($t = 4.17$, d.f. = 148 , $p < 0.000$). 60% of stroke subjects had a tHcy level of ≥ 15 μmol/l compared to 25% of control subjects. For the entire sample, 31% of stroke and 10% of control subjects were in the upper quartile of tHcy values. tHcy levels were significantly correlated with age ($r = 0.25$, $p = 0.003$ for whole group), folate (–0.21, $p < 0.02$) and creatinine ($r = 0.59$, $p = 0.000$) levels, but not with B_{12} levels. The two groups did not differ significantly on folate or serum B_{12} levels, but the stroke group had significantly higher creatinine levels ($p = 0.001$). tHcy levels were higher in men (17.2 ± 6.9 μmol/l) compared to women (14.9 ± 6.5 μmol/l), but when corrected for age, creatinine and folate levels, the estimated marginal mean for tHcy in men was 15.8 μmol/l and in women 16.1 μmol/l ($p = 0.78$). The stroke sample performed less well on all domains of neuro-psychological functioning. The stroke sample had higher signal hyperintensity scores on T_2-weighted MRI in both the deep white matter and periventricular regions, but the VBRs were not significantly different for the two groups. These results are presented in table 1. We compared the subjects who received an MRI scan (n = 97) with those who did not (n = 53) on age, gender, education and tHcy levels. There were no significant differences between the groups.

Hcy and Cognition

The tHcy levels had a significant correlation with all four domains of cognitive functioning for the entire sample, and these were also significant after correction for age, folate and creatinine levels, for the domains of executive functioning and attention. The correlations suggested poorer cognitive functioning with higher tHcy levels. This was confirmed by testing the relationship between tHcy levels and the first cognitive factor of a principal component reduction of the neuropsychological data. Forty-three percent of cognitive variance was explained by this first PCA factor (PCA1) alone. When scores on the first factor were used to divide the entire sample into high and low cognitive groups, being in the pathological tHcy range increased odds of membership of the lower cognitive group by 3.3 times (controlling for age, folate and creatinine, OR = 3.27, 95% confidence interval = 1.30–8.24). A linear association was also found between tHcy and PCA1: $r = –0.30$, $p = 0.001$ after controlling for age, folate, B_{12} levels and creatinine. The high and low cognitive groups were divergent in tHcy distribution (independent sample t test: $t = 2.9$, d.f. = 141, $p = 0.005$).

When examining the two groups separately, tHcy levels were significantly related to executive functioning in the stroke group and to memory functioning in the control group. After controlling for age, folate and creatinine levels, the correlation of tHcy with frontal-executive functioning approached significance (table 2). Since the magnitude of the relationship was small (between $r = 0.2$ and 0.3), sample sizes of individual groups require approximately 125 subjects to achieve 80% power while testing significance at the 0.05 level. We examined the relationship of tHcy levels to performance on individual neuropsychological tests, and the best correlation was with Trails B time ($r = 0.28$, $p = 0.003$ for the whole group; $r = 0.30$, $p = 0.013$ for the stroke group), which was significant after controlling for age, folate and creatinine levels.

Furthermore, there was evidence of a graded relationship between tHcy and cognitive performance. The whole sample was divided into tHcy quartiles and cognitive correlations tested. Table 3 shows that these associations

Table 3. Graded relationship between Hcy and other variables (controlling for age)

Hcy quartile, µmol/l range	TMT-B (n = 34/quartile)	Factor 1 (n = 37/quartile)	DWMHs (n = 24/quartile)
1 (6–12.0)	0.23 (0.201)	–0.12 (0.478)	0.01 (0.973)
2 (12–14.75)	0.18 (0.291)	–0.18 (0.273)	0.28 (0.184)
3 (14.75–19.0)	0.36 (0.054*)	–0.04 (0.825)	–0.14 (0.465)
4 (19.0+)	0.46 (0.008)	–0.24 (0.155)	0.013 (0.957)

Relationships marked by * are significant if using one-tailed test.
TMTB = Trail Making Test B Time.
Factor 1 = Neuropsychological Factor 1 score (see text).
DWMHs = Deep white matter hyperintensity score.

Table 4. Zero order and partial correlations between serum tHcy and MRI brain measurements, broken down by group

Correlate	Whole group (n = 97)	Controls (n = 42)	Stroke patients (n = 55)
Zero order			
Infarcts	0.43 (<0.001)	–0.09 (0.561)	0.30 (0.035)
Total stroke volume	0.24 (0.02)	–0.21 (0.192)	0.01 (0.362)
VBR [1] anterior	0.14 (0.188)	0.44 (0.003)	0.03 (0.825)
midsection	0.20 (0.05)	0.49 (0.001)	0.01 (0.940)
Whole brain hyperintensity score [2]	0.2 (0.05)	–0.01 (0.940)	0.15 (0.269)
PVH score[2]	0.16 (0.123)	0.041 (0.798)	0.14 (0.309)
DWMH score[2]	0.22 (0.033)	–0.01 (0.940)	0.16 (0.250)
Internal capsule hyperintensity score[2]	0.23 (0.022)	0.04 (0.798)	0.15 (0.275)
Controlling for age, folate and creatinine			
VBR [1] anterior	0.26 (0.018)	0.48 (0.003)	0.24 (0.132)
midsection	0.22 (0.051)	0.49 (0.002)	0.04 (0.826)
Whole brain hyperintensity score[2]	0.01 (0.898)	–0.35 (0.038)	–0.02 (0.899)

p values are given within parentheses.
* All correlations are Pearson's except for number of infarcts, for which Spearman's ρ was used.
[1] Ventricle to brain ratio (see text).
[2] Summary ratings of T_2-weighted hyperintensities (see text).

were strongest in the highest tHcy quartile and absent in the physiological range.

Mechanism of the Relationship

The mechanism of the relationship between tHcy and cognition was explored by examining its association with neuroimaging parameters (table 4). tHcy had a significant correlation with number of infarcts (Spearman's ρ = 0.3, p = 0.04) in the stroke group. To determine if tHcy concentration was related to individual stroke size, the average stroke volume was categorized into quintiles. The categories did not differ significantly in mean tHcy concentrations, suggesting that the association of tHcy was with stroke number and not size. The correlation with DWMH score was significant (r = 0.23, p = 0.04) for the entire group, but this was not significant after correcting for age and gender. There was a weak association between tHcy and VBR, midsection, in the whole group (r = 0.21, p = 0.07). When the groups were examined separately, there was a strong age, gender, folate and creatinine invariant relationship between tHcy and VBR for the control group only (r = 0.54, p = 0.002). The best neuroimaging correlates of cognitive performance (factor 1 scores) were DWMH (r = –0.40, p = 0.000), stroke number (r = –0.45,

p = 0.000) and stroke volume (r = –0.47, p = 0.000). Each of these was significant after adjusting for age and gender. Only DWMH was associated with TMT-B performance independent of age and Hcy levels. A comparison of the high and low cognitive performance groups, divided into two halves on the basis of their PCA1 scores, of the biochemical, neuroimaging and neurochemical parameters showed that the low cognitive group had higher tHcy levels (17.3 μmol/l cf. 14.4 μmol/l, t = 2.7, p = 0.007) as well as more T_2-weighted hyperintensities (OR = 2.46, p < 0.001).

A multiple regression analysis showed that there was a small direct effect of tHcy on cognition in the stroke group (β = –0.017, p = 0.08) when age, stroke number, stroke volume and WMH scores had been accounted for.

Discussion

Elevated tHcy levels have been associated with cerebrovascular disease in a large number of studies. In a recent meta-analysis of the literature, which included eight cross-sectional and four longitudinal studies, Moller et al. [10] reported that with tHcy levels higher than the 95th percentile, the OR of cerebrovascular disease was 3.97. Our study supports an association of mild HHcy with stroke, with our stroke subjects having elevated mean tHcy levels compared with control subjects. Low serum folate and B_{12} levels, high creatinine levels or age of subjects could not account for these elevated levels.

HHcy has been more closely linked with microvascular rather than macrovascular brain disease [44]. It is important to note the characteristics of our stroke patients. The mean volume of infarcted tissue on T_1-weighted MRI, performed 3–6 months after the stroke, was 1.5 ml, suggesting that the majority of our subjects had small strokes. Some of the control subjects (n = 4, 5%) also had 'silent' lacunar infarcts, with a mean volume of infarction in this group being 0.052 ml. All subjects had some degree of small vessel disease as evidenced by white matter lesions on MRI. For this reason, we pooled the data from the two groups for some of the associations. Our subjects did not show a significant relationship between tHcy levels and ratings of DWMHs after correction for age. This is in contrast with Fassbender et al. [44] and Evers et al. [11] who reported an association of high Hcy levels with microangiopathy. There was however a significant relationship with VBR as a marker of cerebral atrophy, especially in the control group, which was independent of the covariates examined (folate and creatinine levels and age). This result has been previously reported [45]. Since many of the correlations examined using the entire sample were in the range of 0.2–0.3, we cannot rule out the possibility of false-negative results because of a small sample size for the individual groups. With this type of association, a power estimate for the stroke group was approximately 0.77.

There are a number of limitations of our data. First, this is a cross-sectional study, from which only plausible conclusions can be drawn about the direction of causality, that must be further examined in a longitudinal design. Second, the sampling for tHcy was performed within 1–2 weeks of the stroke, whereas the MRI was performed about 4 months later. Since none of the subjects had a further stroke in the intervening period, it is likely that the MRI scan at 4 months would accurately reflect the cerebrovascular disease at baseline. We were unable to repeat the tHcy levels at 4 months, which would establish a more stable tHcy level in these subjects. It is possible that our measurements of tHcy were affected by an acute phase reaction. It has been reported that tHcy levels have an acute phase reduction and possibly a post-acute phase increase for different reasons [46]. An acute phase reduction would, however, have produced a result opposite to the one we report in our paper. It therefore does not weaken the findings of this study. Third, the sample size was modest for some of the associations, such that some associations were significant for the entire sample but not so when groups were examined separately. We justify pooling the subjects for examining some of the associations because of shared features such as DWMHs, silent strokes and brain atrophy, and the observation that trends were similar in the two groups. For such analyses, we accounted for infarcts in the brains of the stroke patients. Fourth, the control sample was not a random community sample. However, we tried to recruit controls from the same neighbourhood as the patients. The lack of matching on gender and baseline intellectual functioning was dealt with by using these two variables as covariates in the analyses. Fifth, not all subjects had MRI scans because of refusals or ineligibility, dealt with by restricting the regression analyses to a limited number of variables, as suggested by the preliminary analyses. We compared the subjects with and without MRIs on sociodemographic and neuropsychological variables, on which they did not differ statistically, arguing against a biased selection of subjects for MRI.

The most notable finding of our study is the relationship between cognitive functioning and tHcy levels. These were significant for the entire group for executive func-

tioning and memory, after correction for age, and folate, vitamin B_{12} and creatinine levels. In fact, when the relationship with individual tests was examined, it was strongest with the time taken to complete Trails B [37], which was significant for the stroke group as well as the entire sample. In addition to being a visuomotor tracking task, this test examines the ability of the individual to shift set from one sequence to the other, and is very sensitive to brain damage with disturbance in frontal-executive functioning. In our sample, performance on Trails B correlated significantly with PVH and DWMH scores, suggesting that white matter disease may be one mediating factor in this relationship. However, that does not entirely explain the relationship between elevated tHcy levels and test performance, as indicated by the regression analysis, and other effects of Hcy on cognition should be considered.

There has been a recent interest in the role HHcy may play in neurodegeneration. The relationship between Hcy levels and cognitive impairment has been reported in two large epidemiological studies of healthy subjects [13, 47], with the association not being accounted for by low vitamin B_{12}, B_6 or folate levels. In the study by Riggs et al. [13], Hcy was found to correlate with performance on a spatial copying task. Recently, three case-control studies [14, 15, 48] and one prospective study [16] have reported a relationship between high Hcy levels and AD. These findings suggest that the impact of Hcy on cognition may not be exclusively mediated through vascular disease. Our finding of the relationship with cognitive functioning which could not be accounted for by low vitamin levels or MRI-documented vascular disease supports this conclusion. Further support for this comes from the relationship between tHcy levels and brain atrophy in the control sample. Of course, it is possible that Hcy is related to microvascular disease that is not detected by T_2-weighted MRI, and neuropathological examination is necessary to determine this.

The association of Hcy with cognitive deficits does not necessarily imply that HHcy causes the impairment. The possibility that cognitive impairment led to vitamin deficiency and a secondary rise in Hcy levels in these patients was, however, considered unlikely. None of the subjects was deficient in folate and B_{12}, and we did not see a significant correlation between Hcy and vitamin B_{12} levels. The mechanism by which Hcy may cause cognitive impairment not mediated through vascular disease is not understood, but a number of possibilities have been suggested [49]. Hcy has been reported to be directly neurotoxic through its action on the N-methyl-*D*-aspartate glutamate receptor [50], and its metabolite homocysteic acid is also excitotoxic [51]. Exposure of rat hippocampal neurons to Hcy has been shown to lead to activation of poly-ADP-ribose polymerase and NAD depletion, which precede mitochondrial dysfunction, oxidative stress, caspase activation and neuronal apoptosis [46]. It is also possible that Hcy-related microvascular disease increases the deposition of β-amyloid plaques and neurofibrillary tangles that are associated with AD.

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References

1 McCully KS: Vascular pathology of homocysteinemia: Implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111–128.
2 Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I: Hyperhomocysteinemia: An independent risk factor for vascular disease. N Engl J Med 1991;324:1149–1155.
3 Kang SS, Wong PW, Malinow MR: Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. Annu Rev Nutr 1992;12:279–298.
4 Mayer EL, Jacobsen DW, Robinson K: Homocysteine and coronary atherosclerosis. J Am Coll Cardiol 1996;27:517–527.
5 Ueland PM, Refsum H: Plasma homocysteine – A risk factor for vascular disease: Plasma levels in health, disease, and drug therapy. J Lab Clin Med 1989;114:473–501.
6 Welch GN, Loscalzo J: Homocysteine and atherosclerosis. N Engl J Med 1998;338:1042–1050.
7 Stein JH, McBride PE: Hyperhomocysteinemia and atherosclerotic vascular disease: Pathophysiology, screening and treatment. Arch Intern Med 1998;158:1301–1306.
8 Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom Le, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Carcon D, Andria G: Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA 1997;277:1775–1781.

9 Perry IJ, Refsum H, Morris RW, Ebrahim SB, Ueland PM, Shaper AG: Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. Lancet 1995;346:1395–1398.
10 Moller J, Nielsen GM, Tvedegaard KC, Andresen NT, Jorgensen PE: A meta-analysis of cerebrovascualr disease and hyperhomocysteinemia. Scand J Clin Lab Invest 2000;60:491–499.
11 Evers S, Koch HG, Grotemeyer KH, Lange B, Deufel T, Ringelstein EB: Features, symptoms, and neurophysiological findings in stroke associated with hyperhomocysteinemia. Arch Neurol 1997;54:1276–1282.
12 Diaz-Arrastia R: Hyperhomocysteinemia: A new risk factor for Alzheimer's disease? Arch Neurol 1998;55:1407–1408.
13 Riggs KM, Spiro A 3rd, Tucker K, Rush D: Relations of vitamin B12, vitamin B6, folate, and homocysteine to cognitive performance in the Normative Aging Study. Am J Clin Nutr 1996;63:306–314.
14 Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM: Folate, vitamin B_{12}, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55: 1449–1455.
15 Lehmann M, Gottfries CG, Regland B: Identification of cognitive impairment in the elderly: Homocysteine is an early marker. Dement Geriatr Cogn Disord 1999;10:12–20.
16 Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, WolfPA: Plasma homocysteine as a risk factor for dementia and Alzheimer's Disease. N Engl J Med 2002;346:476–483.
17 Foulkes MA, Wolf PA, Price TR, Mohr JP, Hier DB: The stroke data bank: Design, methods, and baseline. Stroke 1988;19:547–554.
18 Folstein MF, Folstein SE, McHugh PR: 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–198.
19 Nexo E, Engbaek F, Ueland PM, Westby C, O'Gorman P, Johnston C, Kase BF, Guttormsen AB, Alfheim I, McPartlin J, Smith D, Moller J, Rasmussen K, Clarke R, Scott JM, Refsum H: Evaluation of novel assays in clinical chemistry: Quantification of plasma total homocysteine. Clin Chem 2000;46:1150–1156.
20 Wang XL, Duarte N, Cai H, Adachi T, Sim AS, Cranney G, Wilcken DE: Relationship between total homocysteine polymorphisms of homocysteine metabolism related enzymes, risk factors and coronary artery disease in the Australian hospital-based population. Atherosclerosis 1999;146:133–140.
21 Nelson HE, Willison J: National Adult Reading Test (NART): Test Manual, ed 2. Windsor, NFER Nelson, 1991.
22 Yesavage JA, Brink TL, Rose TL, Lim O, Huang V, Adey M, Leirer VO: Development and validation of a geriatric depression screening scale: A preliminary report. J Psychiatr Res 1982–83;17:37–49.
23 Briggs GG, Nebes RD: Patterns of hand preference in a student population. Cortex 1975;11: 230–238.
24 Wechsler D: Wechsler Adult Intelligence Scale, revised. New York, Psychological Corporation, 1981.
25 Smith A: Symbol Digit Modalities Test. Los Angeles, Western Psychological Services, 1991.
26 Wechsler D: Wechsler Memory Scale, revised. San Antonio, Psychological Corporation, 1987.
27 Kaplan EF, Goodglass H, Weintraub S: The Boston Naming Test, ed 2. Philadelphia, Lea & Febiger, 1978.
28 Mack WJ, Freed DM, Williams BW, Henderson VW: Boston Naming Test: Shortened version for use in Alzheimer's disease. J Gerontol 1992;47:164–168.
29 DeRenzi E, Vignolo LA: The Token Test: A sensitive test to detect disturbances in aphasics. Brain 1962;85:665–678.
30 Spreen O, Strauss E: A Compendium of Neuropsychological Tests. Administration, Norms and Commentary, ed 2. New York, Oxford University Press, 1998.
31 Strub RL, Black FW: Mental Status Examination in Neurology, ed 2. Philadelphia, Davis, 1985.
32 Benton AL, Hamsher K deS, Varney NR, Spreen O: Contributions to Neuropsychological Assessment. New York, Oxford University Press, 1983.
33 Kertesz A: Western Aphasia Battery. San Antonio, Psychological Corporation, 1982.
34 Mattis S: Dementia Rating Scale (DRS). Odessa, Psychological Assessment Resources, 1988.
35 Goldstein KH, Sheerer M: Abstract and concrete behaviour: An experimental study with special tests. Psycholog Monogr 1941;53:239.
36 Tombaugh TN, Kozak J, Rees L: Normative data for the controlled oral word association test, pers commun, 1996; in Spreen and Strauss [30].
37 Reitan RM, Wolfson D: The Halstead-Reitan Neuropsychological Test Battery. Tucson, Neuropsychology Press, 1985.
38 Ivnik RJ, Malec,JF, Smith GE, Tangelos EG, Peterson EG, Kokmen E, Kurland LT: Mayo's Older Americans Normative Studies: WAIS-R norms for ages 56 to 97. Clin Neuropsychol 1992;6(suppl):1–30.
39 Ivnik RJ, Malec JF, Smith GE, Tangelos EG, Peterson EG, Kokmen E, Kurland LT: Mayo's Older Americans Normative Studies: WMS-R norms for ages 56 to 94. Clin Neuropsychol 1992;6(suppl):49–82.
40 Ivnik RJ, Malec JF, Smith GE: Neuropsychological tests' norms above age 55: COWAT, BNT, MAE Token, WRAT-R Reading, AMNART, STROOP, TMT and JLO. Clin Neuropsychol 1996;10:262–278.
41 Ryan JJ, Paolo AM: A screening procedure for estimating premorbid intelligence in the elderly. Clin Neuropsychol 1992;6:53–62.
42 Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA: MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. AJR Am J Roentgenol 1987;149:351–356.
43 Victoroff J, Mack WJ, Grafton ST, Schreiber SS, Chui HC: A method to improve interrater reliability of visual inspection of brain MRI scans in dementia. Neurology 1994;44:2267–2276.
44 Fassbender K, Mielke O, Bertsch T, Nafe B, Froschen S, Hennerici M: Homocysteine in cerebral macroangiography and microangiopathy. Lancet 1999;353:1586–1587.
45 Sachdev P, Valenzuela M, Wang XL, Looi JCL, Brodaty H: Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. Neurology 2002; 58:1539–1541.
46 Lindgren A, Brattstrom L, Norrving B, Hultberg B, Andersson A, Johansson BB: Plasma homocysteine in the acute and convalescent phases after stroke. Stroke 1995;26:795–800.
47 McCaddon A, Davies G, Hudson P, Tandy S, Cattell H: Total serum homocysteine in senile dementia of Alzheimer type. Int J Geriatr Psychiatry 1998;13:235–239.
48 Diaz-Arrastia R: Homocysteine and neurologic disease. Arch Neurol 2000;57:1422–1427.
49 Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, Raydu PV, Arnelle DR, Stamler JS: Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci USA 1997;94: 5923–5928.
50 Beal MF, Swartz KJ, Finn SF, Mazurek MF, Kowall NW: Neurochemical characterization of excitotoxin lesions in the cerebral cortex. J Neurosci 1991;11:147–158.
51 Kruman II, Culmsee C, Chan SL, Kurman Y, Guo Z, Penix L, Mattson MP: Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 2000;20:6920–6926.