Folate, Vitamin B_{12}, and Serum Total Homocysteine Levels in Confirmed Alzheimer Disease

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Background: Recent studies suggest that vascular disease may contribute to the cause of Alzheimer disease (AD). Since elevated plasma total homocysteine (tHcy) level is a risk factor for vascular disease, it may also be relevant to AD.

Objective: To examine the association of AD with blood levels of tHcy, and its biological determinants folate and vitamin B_{12}.

Design: Case-control study of 164 patients, aged 55 years or older, with a clinical diagnosis of dementia of Alzheimer type (DAT), including 76 patients with histologically confirmed AD and 108 control subjects.

Setting: Referral population to a hospital clinic between July 1988 and April 1996.

Main Outcome Measures: Serum tHcy, folate, and vitamin B_{12} levels in patients and controls at entry; the odds ratio of DAT or confirmed AD with elevated tHcy or low vitamin levels; and the rate of disease progression in relation to tHcy levels at entry.

Results: Serum tHcy levels were significantly higher and serum folate and vitamin B_{12} levels were lower in patients with DAT and patients with histologically confirmed AD than in controls. The odds ratio of confirmed AD associated with a tHcy level in the top third (≥14 µmol/L) compared with the bottom third (≤11 µmol/L) of the control distribution was 4.5 (95% confidence interval, 2.2-9.2), after adjustment for age, sex, social class, cigarette smoking, and apolipoprotein E $\epsilon 4$. The corresponding odds ratio for the lower third compared with the upper third of serum folate distribution was 3.3 (95% confidence interval, 1.8-6.3) and of vitamin B_{12} distribution was 4.3 (95% confidence interval, 2.1-8.8). The mean tHcy levels were unaltered by duration of symptoms before enrollment and were stable for several years afterward. In a 3-year follow-up of patients with DAT, radiological evidence of disease progression was greater among those with higher tHcy levels at entry.

Conclusions: Low blood levels of folate and vitamin B_{12}, and elevated tHcy levels were associated with AD. The stability of tHcy levels over time and lack of relationship with duration of symptoms argue against these findings being a consequence of disease and warrant further studies to assess the clinical relevance of these associations for AD.

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ALZHEIMER DISEASE (AD) and vascular dementia—the 2 major subtypes of dementia—have distinct pathological features, but these frequently coexist,[1] and the combination results in more severe symptoms of dementia.[2,3] In addition to the established role of cerebral infarction in vascular dementia,[4] recent studies have suggested that cardiovascular disease,[5,6] atherosclerosis, and abnormalities in the cerebral microvasculature[7-10] may also be relevant to the cause of AD. Furthermore, the $\epsilon 4$ allele of apolipoprotein E (apoE) is a risk factor not only for AD[11] but also for cardiovascular disease, and the presence of both may interact in the cause of AD.[7,12]

These findings have prompted us to investigate whether elevated serum total homocysteine (tHcy) levels, a risk factor for vascular disease,[13,14] may also be relevant to AD. Moderately elevated levels of tHcy are common in the population and increase with aging,[15,16] and have been reported in patients with clinical diagnoses of dementia of Alzheimer type (DAT) or vascular dementia.[17,18] In the present study, we examined the association of histologically confirmed AD with serum tHcy levels using a case-control design. We studied the nutritional (folate and vitamin B_{12})[15]

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SUBJECTS AND METHODS

SUBJECTS AND CLINICAL INVESTIGATIONS

Between July 1988 and April 1996, 228 patients from the Oxfordshire Health Authority area in England who had varying degrees of cognitive dysfunction were referred to the Oxford Project to Investigate Memory and Ageing (OPTIMA).[21] Patients younger than 55 years (n = 9) or for whom blood samples were not available for tHcy measurements (n = 28) were excluded.

Among the 191 remaining patients, 76 of the 103 on whom an autopsy was performed had a histological diagnosis of AD using Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria[22] for definite or probable AD, 12 had vascular dementia, and 15 had other causes of dementia. A further 88 living patients had a clinical diagnosis of probable or possible DAT according to National Institute of Neurological Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria,[23] and these were combined with the 76 histopathologically confirmed AD cases to give 164 patients with a clinical diagnosis of DAT. Among the AD cases, histological evidence of concomitant cerebrovascular disease was defined by the presence of 1 or more infarcts in the cortex, thalamus, or basal ganglia in addition to the typical histological features of AD. These patients were compared with 108 elderly volunteer controls without symptoms of memory impairment (17 of whom were patients' relatives) who were recruited by leaflets or by lectures given at retirement association clubs or from general practices in the Oxfordshire Health Authority area during the same period. All subjects underwent a detailed clinical history, physical examination, assessment of cognitive function (Cambridge Examination for Mental Disorders of the Elderly [CAMDEX],[24] from which the Cambridge Cognitive Examination [CAMCOG] and Mini-Mental State Examination [MMSE] scores were derived) annually. X-ray cranial computed tomography scans were performed annually using both the standard axial angle and the temporal lobe–oriented angle, as described previously.[21] The minimum thickness of the medial temporal lobe at the level of the brainstem was measured from hard copies of the temporal lobe–oriented scan by 2 independent observers who were unaware of the diagnosis and previous scans. A Dementia Severity Rating score was derived from the CAMDEX at the time of assessment and scored as follows: none, 0; minimal, 0.5; mild, 1; moderate, 2; and severe, 3. Informed consent was obtained for all individuals to participate in the study, which had local ethics committee approval.

BIOCHEMICAL MEASUREMENTS

Nonfasting blood samples were taken at the first visit and stored at −70°C. Vitamin B_{12} level was measured using a radioimmunoassay; serum folate and red blood cell folate levels were determined by microbiological assays on fresh samples. Levels of tHcy were determined by high-performance liquid chromatography with fluorescence detection.[25] The intraclass correlation coefficient of tHcy between replicate samples taken at 2-month intervals on 7 occasions during a 1-year period in 96 healthy elderly subjects was 0.88.[26] ApoE genotypes[27] and the 677C→T mutation in the *MTHFR* gene[19] were determined by standard methods. All measurements were obtained in a blind manner to each other and to the diagnoses.

STATISTICAL ANALYSES

The odds ratio (OR) of AD was examined using logistic regression analysis for the top third and middle third compared with the bottom third of the tHcy concentration distribution in the control population. For folate and vitamin B_{12}, the OR of AD for the bottom third and middle third were compared with the top third of the distribution in the control population. In the regression analysis models, age and years of full-time education were entered as continuous variables, and sex, social class (manual or nonmanual employment), cigarette smokers (ever or never smokers), and apoE $\epsilon 4$ allele status (present or absent) were entered as dichotomous variables. The confidence interval (CI) for each OR was estimated by treating these as "floating absolute risks."[28]

and genetic (*MTHFR* [methylenetetrahydrofolate reductase] polymorphisms)[19] determinants of tHcy levels, and their relation to AD. We also assessed whether differences in tHcy levels at study recruitment were related to the clinical course by using atrophy of the medial temporal lobe as a marker of disease progression.[20]

RESULTS

STUDY POPULATIONS

Characteristics of the study populations are shown in **Table 1**. The clinically diagnosed DAT patients and controls were well matched for age, sex, and smoking status (Table 1). The subset of patients with histologically confirmed AD were older than the controls, and both case populations had a lower social class distribution than controls. The disease severity among the patients is reflected by the low cognitive scores (MMSE and CAMCOG). Fifty-nine (36%) of the patients with clinically diagnosed DAT and 43 (57%) of the patients with histologically confirmed AD had a Dementia Severity Rating score (maximum 3) of 2 or greater at the first visit. Forty-one (25%) of the patients with clinically diagnosed DAT and histologically confirmed AD were residents in institutions at the first visit. Among the patients with histologically confirmed AD, the median interval between the first visit and death was 29 months (95% CI, 2-69 months).

SERUM HOMOCYSTEINE

The mean serum tHcy levels at the first visit were significantly higher in patients with clinically diagnosed DAT and histologically confirmed AD than in controls (Table 1). The cumulative frequency plots (**Figure**) show a shift in the distribution of tHcy concentrations

Table 1. Characteristics at Presentation in Controls and in Patients With Alzheimer Disease*

Variable	Controls (n = 108)	Patients With Alzheimer Disease: Clinically Diagnosed (n = 164)	Patients With Alzheimer Disease: Histologically Confirmed (n = 76)
Clinical variables			
Age, mean (SD), y	72.8 (8.8)	73.2 (8.6)	76.6 (8.0)†
Sex, % male	43	39	37
Current smokers, %	21	21	24
Social class: grades 1 and 2, %	80	46†	49†
Full-time education, mean (SD), y	11.4 (1.5)	10.4 (2.0)†	10.3 (2.3)†
CAMCOG score (maximum 107), mean (SD)	97.8 (4.9)	55.2 (26.5)†	45.1 (27.5)†
MMSE score (maximum 30), mean (SD)	28.5 (1.7)	16.2 (8.0)†	12.8 (8.1)†
Minimum medial temporal lobe thickness, mean (SD), mm	13.5 (3.0)	9.9 (2.9)†	9.3 (2.9)†
Biochemical variables, mean (SD)			
Total homocysteine, µmol/L	13.2 (4.0)	15.3 (8.4)‡	16.3 (7.4)†
Serum folate, nmol/L	22.9 (10.0)	17.6 (10.7)†	15.2 (9.5)†
Red blood cell folate, nmol/L	991 (407)	866 (446)‡	737 (386)†
Vitamin B_{12}, pmol/L	253 (100)	236 (112)	215 (79)‡
Creatinine, µmol/L	93 (19)	90 (18)	90 (20)
Albumin, g/L	45 (4)	43 (3.8)†	42 (4)†
Hemoglobin, g/L	136 (15)	133 (14)	131 (15)‡
Genotypes			
ApoE ε4 allele frequency, %	14	38†	44†
MTHFR homozygous mutant frequency, %	9	7	5

*CAMCOG indicates Cambridge Cognitive Examination; MMSE, Mini-Mental State Examination; ApoE, apolipoprotein E; and MTHFR, methylenetetrahydrofolate reductase.

†*P*<.001 vs controls.

‡*P*<.05.

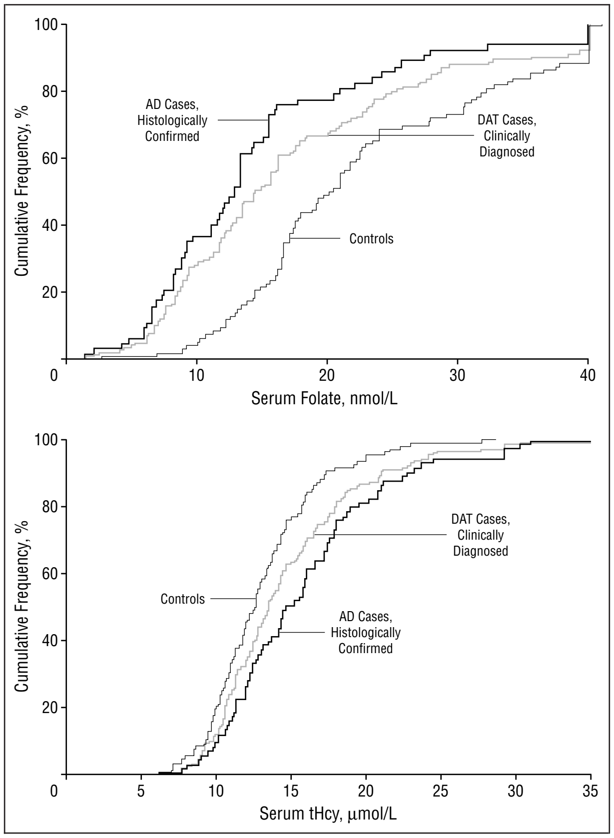
to higher values in patients with clinically diagnosed DAT and histologically confirmed AD compared with controls. Seventy-four patients (45%) with clinically diagnosed DAT and 45 patients (59%) with histologically confirmed AD had a tHcy value in the top third (≥14 µmol/L) of the control distribution.

To identify possible confounders for this association, we looked at the influence of several variables on tHcy levels. Among controls, tHcy level was positively correlated with age ($r = 0.32$, $P = .001$), male sex ($r = 0.27$, $P = .004$), cigarette smoking ($r = 0.23$, $P = .02$), and creatinine level ($r = 0.44$, $P < .001$), and was inversely associated with levels of serum folate ($r = -0.34$, $P = .001$), vitamin B_{12} ($r = -0.32$, $P = .002$), and red blood cell folate ($r = -0.21$, $P = .05$). There was no association between tHcy and apoE ε4, or the *MTHFR* mutation, or with social class or years of full-time education in the control population. Among the DAT cases, years of full-time education were inversely related with social class grades 1 to 6 ($r = -0.58$, $P < .001$), but tHcy levels were unrelated to social class ($r = 0.03$, $P = .72$) or years of full-time education ($r = -0.10$, $P = .28$). The mean (SD) tHcy levels were 15.5 (7.5) µmol/L in social class grades 1 and 2 and 15.1 (9.0) µmol/L in grades 3 to 6.

Table 2 shows that the associations of tHcy levels with both clinically diagnosed DAT and histologically confirmed AD were independent of age, sex, smoking status, apoE ε4, and social class. When social class was replaced by years of education in the multivariate analysis, an OR of 4.6 (95% CI, 1.8-5.5) was found for histologically confirmed AD in subjects with tHcy levels in the top third of the distribution; the OR was 3.6 (95% CI, 1.7-5.7) when both social class and years of education were adjusted for simultaneously. In addition, when the analyses were confined to cases (n = 38) and controls (n = 88) from social classes 1 and 2, the corresponding OR of AD associated with tHcy level in the top third of the distribution was 6.5 (95% CI, 2.6-16.5).

The OR of histologically confirmed AD for the top third compared with the bottom third of tHcy values was 5.1 (95% CI, 1.8-14.0) when both folate and vitamin B_{12} were also included in the regression analysis model. Among the histologically confirmed AD cases, the 31 patients with concomitant histological evidence of cerebrovascular disease had a mean (SD) tHcy level of 16.3 (5.8) µmol/L ($P < .001$ vs controls), and the 45 who had confirmed AD alone had a mean (SD) tHcy level of 16.3 (8.4) µmol/L ($P < .001$ vs controls).

Among the 26 patients with histopathologically diagnosed non-AD dementia, the mean (SD) tHcy level was 20.0 (9.6) µmol/L in 12 with vascular dementia ($P < .001$ vs controls), 18.4 (7.7) µmol/L in 3 with Parkinson disease, 18.7 (7.7) µmol/L in 2 with glioblastoma, 11.0 (3.2) µmol/L in 2 with Huntington disease, and 12.0 (3.2) µmol/L in the 7 remaining patients with various histopathological findings. Two of the 3 patients with Parkinson disease who had elevated tHcy levels were taking levodopa. The OR of vascular dementia associated with a tHcy concentration in the top third (≥14 µmol/L) compared with the bottom third (≤11 µmol/L) of the control distribution was 4.5 (95% CI, 1.6-12.8) after adjustment for age, sex, social class, smoking status, and apoE ε4, which was similar to that for AD.



Cumulative frequency distributions of serum folate (top) and serum total homocysteine (tHcy) (bottom) levels in patients with histologically confirmed Alzheimer disease (AD) and clinically diagnosed dementia of Alzheimer type (DAT) and in control subjects.

SERUM FOLATE AND VITAMIN B_{12}

The mean serum folate and vitamin B_{12} levels at the first visit were significantly lower in AD patients than in controls (Table 1). There was a marked shift in the distribution of folate concentrations to lower values in both clinically diagnosed DAT and histologically confirmed AD patients compared with controls (Figure). Ninety-eight patients (60%) with DAT and 58 patients (76%) with confirmed AD had serum folate concentrations in the bottom third of the control distribution. Among the 12 patients with vascular dementia, the mean (SD) folate levels were 13.8 (11.3) nmol/L ($P = .01$ vs controls) and mean vitamin B_{12} levels were 231.7 (83.4) pmol/L ($P = .52$ vs controls).

Possible confounders of the association of folate level with histologically confirmed AD were also examined. Among the controls, serum folate levels were lower in smokers compared with nonsmokers ($P < .05$). Controls had a higher social class distribution than the patients, but there was no significant difference in folate (or tHcy) concentrations between manual and nonmanual employment classes. After including years of education in addition to social class and all the other confounders shown in Table 2, the OR of AD comparing the bottom third with the top third of serum folate distribution was 2.3 (95% CI, 1.2-4.4). The strength of association between vitamin B_{12} levels and confirmed AD was similar to that for tHcy (Table 2). After the addition of tHcy to the multivariate model, the ORs for confirmed AD for the lower third compared with the upper third of control concentrations of serum folate or vitamin B_{12} were no longer significant: 1.6 (95% CI, 0.8-3.2) and 2.2 (95% CI, 0.8-5.2), respectively.

ApoE AND *MTHFR* POLYMORPHISMS

The apoE $\epsilon 4$ allele frequency was 38% in DAT and 44% in AD cases, compared with 14% in controls. After adjusting for differences in age, sex, smoking status, and social class, the OR of confirmed AD for the presence of 1 or more apoE $\epsilon 4$ alleles compared with none was 7.9 (95% CI, 3.3-18.8). Moreover, the strength of the association of apoE $\epsilon 4$ was unchanged by the inclusion of tHcy in the multivariate analysis. There was no significant difference in the prevalence of the *MTHFR* gene 677C→T mutation, whether expressed as the proportion homozygous (5% vs 9%) or as allele frequency (22% vs 30%), in patients with histologically confirmed AD compared with controls.

INFLUENCE OF DURATION OF MEMORY IMPAIRMENT ON HOMOCYSTEINE AND VITAMIN LEVELS

To assess whether the prior duration of dementia could explain the observed biochemical changes, 72 histologically confirmed AD patients with available data were classified by tertiles of duration of memory impairment (as reported by an informant) before their first visit when the blood samples were taken (**Table 3**). The disease severity was substantially greater in those with a longer duration of memory impairment, but there was no significant trend in the mean levels of any of the biochemical variables with increasing duration of symptoms. The biochemical findings were also unaltered by duration of illness among patients with clinically diagnosed DAT (data not shown).

STABILITY OF HOMOCYSTEINE CONCENTRATIONS OVER TIME

Replicate tHcy measurements were obtained at sequential annual follow-up visits in 30 patients with DAT. The mean tHcy level was 14.1 μmol/L at first visit, 13.6 μmol/L at year 1, 13.6 μmol/L at year 2, and 13.3 μmol/L at year 3, and the correlation coefficients at these intervals with baseline levels were 0.85, 0.83, and 0.78, respectively. Similar estimates of stability in tHcy measurements were obtained in 34 controls with correlation coefficients with baseline tHcy concentrations of 0.78, 0.74, and 0.73 at years 1 through 3, respectively.

HOMOCYSTEINE AND VITAMIN LEVELS AND DISEASE PROGRESSION

To assess whether differences in tHcy and vitamin levels at the first visit were related to disease progression, we compared the results in 43 patients with clinically diagnosed DAT for whom we had computed tomographic scans and MMSE scores from 4 annual visits (**Table 4**).

Table 2. Odds Ratios of Clinically Diagnosed Dementia of Alzheimer Type (DAT) and of Histologically Confirmed Alzheimer Disease (AD) by Total Homocysteine (tHcy) and Vitamin Levels*

	Clinically Diagnosed DAT		Histologically Confirmed AD	
Tertiles	Adjusted for Age and Sex	Adjusted for Age, Sex, Smoking, Social Class, and ApoE ε4	Adjusted for Age and Sex	Adjusted for Age, Sex, Smoking, Social Class, and ApoE ε4
tHcy, μmol/L				
I ≤11.0	1.0 (0.6-1.6)	1.0 (0.6-1.8)	1.0 (0.5-1.9)	1.0 (0.4-2.7)
II 11.1-14.0	1.1 (0.7-1.7)	1.1 (0.7-1.9)	1.3 (0.7-2.3)	1.0 (0.4-2.3)
III >14.0	1.9 (1.2-2.9)	2.0 (1.1-3.4)	3.3 (2.1-5.2)	4.5 (2.2-9.2)
Folate, nmol/L				
III >24.2	1.0 (0.6-1.6)	1.0 (0.5-1.7)	1.0 (0.5-2.1)	1.0 (0.3-3.1)
II 17.2-24.2	0.8 (0.5-1.4)	0.7 (0.4-1.5)	0.6 (0.2-1.6)	0.4 (0.1-1.5)
I ≤17.1	2.5 (1.7-3.8)	2.3 (1.4-3.8)	5.0 (3.1-8.2)	3.3 (1.8-6.3)
Vitamin B_{12}, pmol/L				
III >280	1.0 (0.6-1.6)	1.0 (0.5-1.9)	1.0 (0.5-2.1)	1.0 (0.3-3.8)
II 200-280	1.3 (0.8-2.0)	1.7 (1.0-3.0)	2.1 (1.2-3.6)	5.6 (2.6-11.9)
I ≤199	1.4 (0.9-2.2)	1.4 (0.8-2.5)	1.8 (1.0-3.2)	4.3 (2.1-8.8)

Data are given as odds ratios (confidence intervals). ApoE indicates apolipoprotein E. The confidence intervals for the odds ratios have been estimated by treating these as "floating absolute risks," which take account of the variance in the reference category.[28] The cut points selected were based on the tertile levels in the control subjects.

Table 3. Clinical and Biochemical Variables in Patients With Histologically Confirmed Alzheimer Disease by Duration of Memory Impairment at Presentation*

	Clinical Variables, Mean (SD) or %			Biochemical Variables, Mean (SD)			
Tertiles of Duration of Memory Impairment, y	MMSE Score (Maximum 30)	Minimum Medial Temporal Lobe Thickness, mm	Dementia Severity Rating (Maximum 3) % 2 or 3	Total Homocysteine, μmol/L	Serum Folate, nmol/L	Red Blood Cell Folate, nmol/L	Vitamin B_{12}, pmol/L
I <2	16 (8)	9.9 (2.3)	33	18.8 (10.8)	17.6 (12.2)	749 (466)	216 (82)
II 2-4	14 (8)	9.5 (2.4)	56	15.4 (5.7)	12.9 (5.7)	722 (398)	201 (63)
III >4	8 (6)	8.7 (3.6)	88	14.8 (4.1)	14.0 (7.3)	706 (242)	235 (89)
Test for linear trend *P*	<.001	<.001	<.001	.06	.19	.72	.42

MMSE indicates Mini-Mental State Examination.

At the first visit, the mean age-corrected minimum thickness of the medial temporal lobes in subjects for each of the tertiles of tHcy did not differ. After 3 years, there was significantly greater radiological evidence of disease progression, as assessed by medial temporal lobe thickness, among those with tHcy levels in the middle and upper tertiles compared with those in the lower tertile, who showed little atrophy (Table 4). The association between blood levels of folate and vitamin B_{12} at the first visit and disease progression showed a similar trend, but the differences were not statistically significant (data not shown). The mean (SD) MMSE scores when classified by low, middle, and upper tertiles of tHcy declined from 22 (5), 22 (6), and 19 (8) at the first visit to 13 (6), 15 (9), and 12 (9) after 3 years, but the variance was too large to distinguish any difference from no effect.

COMMENT

Elevated tHcy levels within the range of those associated with vascular disease[14] have been previously reported in patients with clinically diagnosed DAT and in patients with vascular dementia[17,18] and several studies have demonstrated inverse associations between clinically diagnosed DAT and folate and vitamin B_{12} levels.[17,18,29-34] Similar associations also have been shown for cognitive impairment in the elderly.[35] We observed that there were significant associations of histologically confirmed AD and of vascular dementia with moderately elevated blood levels of tHcy and with reduced blood levels of folate and vitamin B_{12}. The cumulative frequency plots (Figure) showed a more marked case-control difference for the distribution of serum folate levels than that for serum tHcy levels, but the relative importance of these associations requires further study. The finding that patients with elevated tHcy levels (Table 4) at the first visit had more rapid atrophy of the medial temporal lobe during a 3-year follow-up than those with lower tHcy levels warrants confirmation in other prospective studies.

The crucial question is whether the observed associations are a cause or consequence of the disease. It could, for example, be argued that dementia leads to a reduced dietary intake of folate and vitamin B_{12}, causing an elevation in tHcy levels. We cannot refute this possibility in this case-control study, but we found no evi-

Table 4. Changes in Minimum Medial Temporal Lobe Thickness (MMTL) Over a 3-Year Period by Total Homocysteine (tHcy) Levels at Presentation in Patients With Clinically Diagnosed Dementia of Alzheimer Type

	Baseline			Follow-up*				
				Ratio of Age-Adjusted Baseline MMTL Values, %				
Tertiles of tHcy in Controls, µmol/L	No. (n = 43)	Absolute MMTL Values, Mean (SD), mm	Age-Adjusted MMTL Values, Mean (SD) (Multiple of Median)†	Year 0	Year 1:0	Year 2:0	Year 3:0	Trend by Years of Follow-up, *P*
I ≤11.0	15	10.0 (2.5)	0.71 (0.16)	100	101	99	95	.07
II 11.1-14.0	11	11.6 (2.7)	0.83 (0.16)	100	93	81	68	.04
III >14.0	17	10.4 (2.5)	0.77 (0.17)	100	97	93	81	.03
Test for difference by tertiles of tHcy‡		.20	.31	. . .	.12	.06	.02	

The follow-up data are adjusted for age and expressed as a ratio of the values at presentation. Ellipses indicate data are not applicable.
†Details of how the age-adjusted values were derived have been previously described.[21]
‡Analysis of variance, probability greater than F. Ellipses indicate data are not applicable.

dence that the duration of memory impairment before the blood sample was taken influenced the biochemical variables. Patients who were symptomatic for more than 4 years before their first visit and whose mean MMSE score was 8 showed no significant difference in the blood levels of tHcy, folate, or vitamin B_{12} from those whose symptoms had been present for less than 2 years and who had a mean MMSE score of 16 (Table 3). Furthermore, the high correlation observed in the DAT cases and controls between baseline tHcy concentrations and those obtained a few years later suggests that the biochemical differences between patients and controls were not due to a progression of the disease during this period. Thus, we suggest that the low vitamin levels and high tHcy levels either existed before the start of AD or developed early in the disease phase. Either way, the abnormality in these biochemical markers may be relevant to the clinical course of AD and should be considered in clinical trials as possible targets for therapeutic intervention. Daily supplementation with 0.5 to 5 mg of folic acid and about 0.5 mg of cyanocobalamin would be expected to reduce homocysteine levels found in typical Western populations on average by about one third.[36] Large-scale clinical trials in high-risk populations are now needed to determine whether lowering blood homocysteine levels reduces the risk of AD and of other dementias.

The chief strength of the present study is the longitudinal assessment of dementia cases with subsequent histopathological confirmation of the types of dementia, so overcoming the inaccuracies of clinical diagnosis. A limitation of this study was that control subjects had a higher overall social class than the patients. However, there were no differences in the mean tHcy or folate levels between manual and nonmanual classes or by years of education in the patients. In addition, when either social class or years of education, or both together, were taken into account in the multivariate analyses, and when the analyses were confined to a subset where most of the controls were recruited, the ORs of AD were still highly significant. A further limitation of this study is the lack of data on recent dietary intake and vitamin supplements in patients compared with controls.

Although the mechanisms underlying the observed associations remain to be established, certain hypotheses should be considered. The association of low folate and vitamin B_{12} levels with AD may be related to their effects on methylation reactions in the brain[37] or may be mediated by their effects on tHcy levels.[15] Homocysteine may have a neurotoxic effect by activating the *N*-methyl-D-aspartate receptor, leading to cell death,[38] or it might be converted into homocysteic acid, which also has an excitotoxic effect on neurons.[39] In addition, elevated tHcy levels are a strong risk factor for vascular disease.[13,14] This might explain the association in the patients with confirmed AD who also had histological evidence of cerebrovascular disease. However, the association with tHcy was also observed in patients with AD and no macroscopic cerebrovascular disease. Perhaps microvascular disease associated with tHcy could play a role in the cause of "pure" AD. We have previously suggested that the onset of AD is triggered by some kind of "insult."[20] This insult could be a consequence of microvascular disease or ischemia in a critical region of the brain, such as the hippocampus, which shows marked vascular abnormalities in AD.[10] The CA_1 pyramidal neurons in the hippocampus are particularly vulnerable to ischemia,[40] and these same neurons show the highest density of neurofibrillary tangles and are selectively depleted in AD.[31,41] Thus, microinfarcts, arising as a consequence of elevated tHcy levels, may result in the deposition of β-amyloid plaques and neurofibrillary tangles that are the pathologic hallmarks of dementia.

Despite the plausible mechanisms, further work is required to establish whether the observed associations are causal. Our data show that elevated tHcy levels and low folate and vitamin B_{12} levels are common in patients with AD. The stability of tHcy levels and lack of relationship with duration of symptoms argue against these associations being a consequence of disease and warrant further studies to determine the relevance of these associations to the onset and progression of AD.

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