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Homocysteine and folate as risk factors for dementia and Alzheimer disease¹⁻³

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ABSTRACT

Background: In cross-sectional studies, elevated plasma total homocysteine (tHcy) concentrations have been associated with cognitive impairment and dementia. Incidence studies of this issue are few and have produced conflicting results.

Objective: We investigated the relation between high plasma tHcy concentrations and risk of dementia and Alzheimer disease (AD) in an elderly population.

Design: A dementia-free cohort of 816 subjects (434 women and 382 men; mean age: 74 y) from an Italian population-based study constituted our study sample. The relation of baseline plasma tHcy to the risk of newly diagnosed dementia and AD on follow-up was examined. A proportional hazards regression model was used to adjust for age, sex, education, apolipoprotein E genotype, vascular risk factors, and serum concentrations of folate and vitamin B-12.

Results: Over an average follow-up of 4 y, dementia developed in 112 subjects, including 70 who received a diagnosis of AD. In the subjects with hyperhomocysteinemia (plasma tHcy > 15 $\mu\text{mol/L}$), the hazard ratio for dementia was 2.08 (95% CI: 1.31, 3.30; $P = 0.002$). The corresponding hazard ratio for AD was 2.11 (95% CI: 1.19, 3.76; $P = 0.011$). Independently of hyperhomocysteinemia and other confounders, low folate concentrations (≤ 11.8 nmol/L) were also associated with an increased risk of both dementia (1.87; 95% CI: 1.21, 2.89; $P = 0.005$) and AD (1.98; 95% CI: 1.15, 3.40; $P = 0.014$), whereas the association was not significant for vitamin B-12.

Conclusions: Elevated plasma tHcy concentrations and low serum folate concentrations are independent predictors of the development of dementia and AD. *Am J Clin Nutr* 2005;82:636-43.

KEY WORDS Homocysteine, dementia, Alzheimer disease, incidence, folate

INTRODUCTION

In Western societies, the prevalence and economic costs of Alzheimer disease (AD) are soaring in step with the increased number of elders in the population (1). Therefore, it is important to identify modifiable risk factors for this disease. The sulfur amino acid homocysteine is a unique candidate for this role because of its direct neurotoxicity (2-4) and its association with cerebrovascular disease (5), which is currently believed to play a

significant role in AD etiology (6). Moreover, elevated concentrations of plasma total homocysteine (tHcy) are an indicator of inadequate folate and vitamin B-12 status (7) and can directly affect brain function via altered methylation reactions (8).

An association between AD and elevated tHcy concentrations has been reported in case-control (9, 10) and cross-sectional (11, 12) studies. Moreover, in nondemented elderly populations, plasma tHcy is inversely associated with poor performance at simultaneously performed tests of global cognitive function (13-15) and specific cognitive skills (13, 16). However, cross-sectional studies cannot determine causality. Only 2 longitudinal studies investigated the relation between hyperhomocysteinemia and risk of incident AD, but their results were inconsistent; the Framingham Study reported a strong association (17), and the Washington Heights-Inwood Columbia Ageing Project (WHI-CAP) reported no association (18). Clarification of this issue is important because consistent evidence of a prospective association between homocysteine and AD would more strongly support the need for intervention trials testing the effectiveness of homocysteine-lowering vitamin therapy in preventing dementia.

Therefore, we examined baseline plasma tHcy in relation to risk of incident dementia and AD in the Conselice Study of Brain Aging (CSBA), an Italian population-based study of older persons.

SUBJECTS AND METHODS

Study population

The CSBA is a population-based survey, already described in detail elsewhere (19, 20), the principal aim of which is to provide

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² Supported by grants from the Italian Ministry of Education, University and Scientific Research, Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica, MIUR, Rome, Italy (60% fund for basic oriented research).

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Received November 9, 2004.

Accepted for publication March 24, 2005.

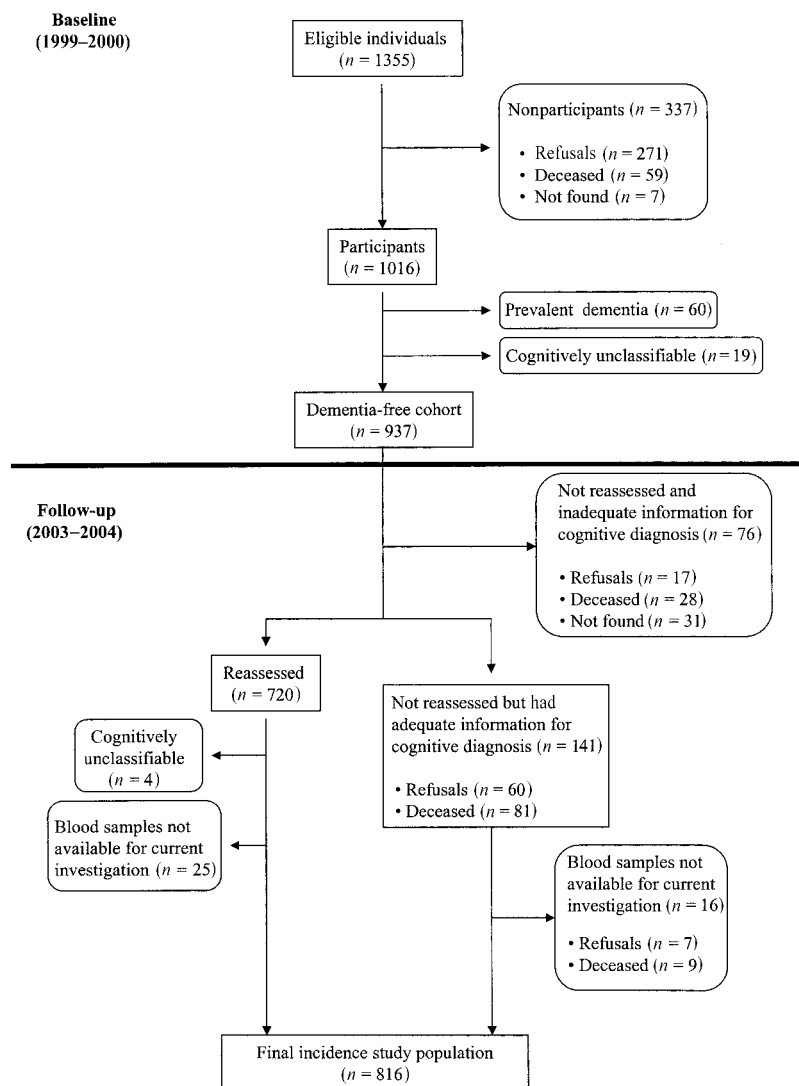


FIGURE 1. Flow chart detailing the derivation of the incidence study sample.

data about epidemiology and risk factors for dementia in the elderly. Its design includes both cross-sectional and longitudinal components. The study was approved by the Institutional Review Board of the Department of Internal Medicine, Cardioangiopathy, and Hepatology, University of Bologna, and written informed consent was obtained from all participants.

Briefly, in 1999–2000, 1016 (75%) of the 1353 individuals aged ≥ 65 y residing in the Italian municipality of Conselice (province of Ravenna, Emilia Romagna region) participated in the prevalence study. Data on cognitive status at the follow-up examination in 2003–2004 were collected for 861 of the 937 participants free of dementia at baseline. A flow chart detailing the derivation of the incidence sample used in this study is reported in **Figure 1**.

Case finding

For 720 survivors who agreed to be reevaluated, the identification of incident cases in 2003–2004 was done by following the same procedure used to identify prevalent dementia at baseline. This consisted of a screening phase and an extensive clinical assessment of those positive at screening to confirm a diagnosis of dementia and to

identify dementia subtype. The Italian version of the Mini-Mental State Examination (MMSE) (21) was used for cognitive screening. Subjects with an MMSE score < 24 were considered positive at screening. To give people with different ages and levels of education an equal probability of being detected as a case, MMSE scores were adjusted by using standardized age and education-specific coefficients previously validated in the Italian population (22). Subjects positive at screening were further cognitively tested with the Mental Deterioration Battery (23), a neuropsychological instrument validated for clinical and epidemiologic use in Italian subjects. Subjects scoring < 10 on the MMSE did not receive further neuropsychological testing, because it was felt that their cognitive status was such that a diagnosis could be made without a more detailed evaluation. Whenever recent neuroradiologic data were not available, subjects were scheduled for a noncontrast computed tomography brain scan. Standardized information on the general functional and mental status of these subjects was also obtained from a collateral informant (a relative or any other person with reliable knowledge of the person, including the subject's medical practitioner). Whenever available, previous medical records were reviewed.

Information from several sources (the subjects themselves, relatives, general practitioners, and death certificates) was considered reliable to define the occurrence or not of dementia for 141 individuals free of dementia at baseline who survived but refused to participate at follow-up or deceased before reexamination in 2003-2004. Informed consent for collection and use of these data was obtained from the subjects themselves or from their relatives, as approved by our Institutional Review Board.

Dementia was defined on the basis of the clinical criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (24). AD was diagnosed on the basis of NINCDS-ADRDA (National Institute of Neurological and Communicative Disease and Stroke-Alzheimer's Disease and Related Disorders Association) criteria for probable or possible AD (25). Diagnoses were independently made by 2 physicians (PF and FM) on the basis of all available information. Finally, a diagnostic conference with a third senior physician (RG) was held to discuss each case.

Baseline data on plasma tHcy, serum B vitamins, and apolipoprotein E genotype were available for 816 persons who were included in the current study (87% of the original dementia-free cohort at baseline).

Laboratory procedures

Baseline venous blood samples were taken after an overnight fast. Blood samples for plasma tHcy measurements were collected in tubes containing EDTA and placed in a refrigerator (-4°C) within 15–30 min of collection. Plasma was separated within 1–3 h, and samples were stored at -70°C for ≤ 12 mo until the analysis was performed. Plasma tHcy concentrations were measured by the fully automatized IMx assay (Abbott Laboratories, Abbott Park, IL). Intra- and interassay CVs were 2.1% and 3.2%, respectively. Serum samples for folate and vitamin B-12 measurements were sent to the biochemical laboratory for immediate immunoelectrochemiluminescence analysis (Elecys Folate Immunoassay and Elecsys B-12 Immunoassay for Elecsys 2010 System, Roche Diagnostics Italia SpA Monza, Milano, Italy). For serum folate, intra- and interassay CVs were 3.1% and 3.8%, respectively. For vitamin B-12, intra- and interassay CVs were 4.3% and 4.6%, respectively. Serum creatinine was measured by the Jaffé method, adapted for autoanalyzers.

Apolipoprotein E genotypes

Genomic DNA was obtained from EDTA-treated blood by using a commercial DNA extraction kit (QiAmp blood kit; Kaga, Crawley, United Kingdom). Apolipoprotein E (APOE) ϵ allele genotyping was performed by polymerase chain reaction as previously described (26). Subjects were divided into 2 groups: those with an APOE $\epsilon 4$ allele and those without an APOE $\epsilon 4$ allele.

Covariates

Potential confounders were defined by using data collected at baseline. Educational status was categorized as 5 or ≥ 6 y of formal education, because only a small number of CSBA participants had completed the 5 y of mandatory education provided for in the old Italian school system. Smoking habit was dichotomized as never smokers, exsmokers, and current smokers. Hypertension was defined as a systolic blood pressure of ≥ 140 mm Hg, a diastolic blood pressure of ≥ 90 mm Hg, or the use of

antihypertensive medication. Diagnoses of diabetes mellitus, cardiovascular diseases (myocardial infarction, angina, peripheral vascular disease, and congestive heart failure), and stroke were based on medical histories provided by the patients and were confirmed by their general practitioner. Whenever available, previous medical records were also reviewed. BMI was calculated as weight in kilograms divided by the square of the height in meters.

Statistical analysis

Variables are presented as means \pm SDs (continuous) or number and percentage (categorical), except for plasma tHcy, serum folate, and serum vitamin B-12 because of their highly skewed distribution. The use of natural log-transformed values provided the best fitting model for the analyses in which they were treated as continuous variables, and values are reported as geometric means and 95% CIs. No standard cutoff exists for hyperhomocysteinemia. Poor B vitamin status and reduced renal function are responsible for a substantial majority of the cases of mild hyperhomocysteinemia in older people (27, 28), but age itself and several genetic, lifestyle, and clinical factors may significantly affect plasma tHcy concentrations (7). For the purposes of this study, as described elsewhere (15), we defined hyperhomocysteinemia as a plasma tHcy concentration > 15 $\mu\text{mol/L}$, corresponding to the 95th percentile among a selected subsample of healthy CSBA participants who were not taking drugs known to affect homocysteine metabolism and had good B vitamin status and normal renal function. Because plasma tHcy concentrations did not significantly differ by sex in this reference population, the same cutoff was used for men and women. This is in agreement with the observation that, even if young men have higher tHcy concentrations than women of the same age, the sex-related difference becomes less with increasing age (7).

Vitamin B concentrations were categorized by using the corresponding median value: low folate was defined as a serum folate concentration ≤ 11.8 nmol/L; low vitamin B-12 was defined as a serum vitamin B-12 concentration ≤ 251 pmol/L. Plasma tHcy and serum folate concentrations were also evaluated with a quartile-based analysis. *t* tests and chi-square tests were used for comparisons between groups. Cox proportional hazards regression models were used to examine the relation between homocysteine (both as a categorical and log-transformed continuous variable) and the incidence of dementia and AD during follow-up, after adjustment for age (in 1-y increment), sex, education, APOE genotype, serum creatinine, B vitamins concentrations (both as categorical and log-transformed continuous variables), and history of stroke. In supplementary analyses, we also adjusted for other potential confounders. For the analyses of incident AD cases, subjects developing other types of dementia were censored at the date of dementia onset (estimated as the midpoint of the time interval from the baseline study until follow-up or death). The statistical analyses were performed by using SYSTAT10 (SPSS Inc, Chicago, IL).

RESULTS

Homocysteine and incidence of dementia and AD

The mean (\pm SD) age of the sample of 816 subjects at baseline was 73.6 ± 6.3 y; 46.8% were men, and the average education



TABLE 1

Comparison of clinical characteristics between subjects with hyperhomocysteinemia and those with normal plasma total homocysteine (tHcy)

	Normal plasma tHcy ($\leq 15 \mu\text{mol/L}$) ($n = 599$)	Hyperhomocysteinemia ($> 15 \mu\text{mol/L}$) ($n = 217$)	P^1
Age (y)	72.6 \pm 5.7 ²	76.1 \pm 7.2	<0.001
Female sex (%)	58.6	61.7	<0.001
Education \geq 6 y	30.4	31.3	0.794
Apolipoprotein E $\epsilon 4$ allele (%)	17.7	12.0	0.050
Serum folate (nmol/L)	13.6 (11.5, 13.9) ³	8.8 (3.6, 21.5)	<0.001
Serum vitamin B-12 (pmol/L)	259 (94, 708)	212 (73, 612)	<0.001
Serum creatinine ($\mu\text{mol/L}$)	83 \pm 16	102 \pm 24	<0.001
Ever smoked (%)	37.1	53.9	<0.001
BMI (kg/m^2)	28.9 \pm 4.6	28.3 \pm 4.2	0.082
History of stroke (%)	2.2	7.4	<0.001
Diabetes (%)	8.5	4.1	0.035
Hypertension (%)	72.3	78.3	0.082
Cardiovascular disease (%)	14.7	25.8	<0.001
Dementia (%)	9.5	25.3	<0.001
Alzheimer disease (%)	6.5	24.3	<0.001

¹ Calculated by using a *t* test (continuous variables) or a chi-square test (categorical variables).² $\bar{x} \pm \text{SD}$ (all such values).³ Geometric \bar{x} ; 95% CI in parentheses (all such values).

level was 4.8 ± 2.4 y. There were 112 cases of incident dementia (70 AD, 34 vascular dementia) according to NINDS-AIREN (National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences) criteria (29), and 8 cases with dementia from other causes) in 3042 person-years of follow-up. Mean follow-up time was 3.8 ± 0.8 y. The overall geometric mean plasma tHcy concentration was 13.0 (95% CI: 6.3, 26.9) $\mu\text{mol/L}$ and ranged from 5.5 to 80 $\mu\text{mol/L}$. Hyperhomocysteinemia was present in 26.6% of the subjects. The baseline characteristics of the subjects with and without hyperhomocysteinemia are presented in **Table 1**. Those with hyperhomocysteinemia were older, more likely to be women, less likely to have an *APOE* $\epsilon 4$ allele genotype or diabetes, and more likely to have low B vitamin status, be a current or exsmoker, and have a history of stroke and cardiovascular disease. Those with hyperhomocysteinemia were also more likely to develop dementia and AD.

The overall results relating hyperhomocysteinemia to the development of any dementia and AD are shown in **Table 2**. Hyperhomocysteinemia was associated with dementia and AD risk even after adjustment for all of the study covariates; this association was not affected by additional adjustment for hypertension, smoking status, diabetes, and BMI. No evidence of interaction was found among the study variables. After adjustment for the study covariates, the hazard ratio (HR) for each increase of 1 SD (0.37) in log-transformed baseline tHcy concentrations was 1.41 (95% CI: 1.17, 1.68; $P < 0.001$) for any dementia and 1.54 (95% CI: 1.24, 1.92; $P < 0.001$) for AD. Examination of the cumulative incidence of dementia (**Figure 2**) and of the adjusted risks of dementia and AD across quartiles of plasma tHcy (**Figure 3**) suggested a dose-related association (dementia: P for trend < 0.001 ; AD: P for trend = 0.002), but HR estimates were statistically significant only for subjects in the top quartile (corresponding to our definition of hyperhomocysteinemia). Alcohol

TABLE 2Cox proportional hazards regression models examining the relation between hyperhomocysteinemia (plasma total homocysteine $> 15 \mu\text{mol/L}$) and risk of any dementia and Alzheimer disease¹

Variables adjusted for	Any dementia		Alzheimer disease	
	HR (95% CI)	P	HR (95% CI)	P
Unadjusted	2.68 (1.85, 3.88)	<0.001	2.22 (1.38, 3.56)	0.001
Age, sex, and education	2.28 (1.53, 3.40)	<0.001	1.81 (1.09, 3.02)	0.022
Age, sex, education, <i>APOE</i> genotype, and creatinine	2.63 (1.73, 4.00)	<0.001	2.20 (1.29, 3.76)	0.004
Age, sex, education, <i>APOE</i> genotype, and serum concentrations of creatinine, folate, and vitamin B-12	2.16 (1.37, 4.00)	<0.001	1.96 (1.09, 3.50)	0.024
Age, sex, education, <i>APOE</i> genotype, stroke, and serum concentrations of creatinine, folate, and vitamin B-12	2.08 (1.31, 3.30)	0.002	2.11 (1.19, 3.76)	0.011
Age; sex, education; <i>APOE</i> genotype; stroke; serum concentrations of creatinine, folate, and vitamin B-12; and additional covariates ²	2.18 (1.37, 3.48)	0.001	2.08 (1.15, 3.79)	0.016

¹ Data are for 816 subjects ($n = 112$ incident dementia cases for any dementia and 70 for Alzheimer disease). HR, hazard ratio.² The additional covariates included smoking status, diabetes, hypertension, cardiovascular disease, and BMI.

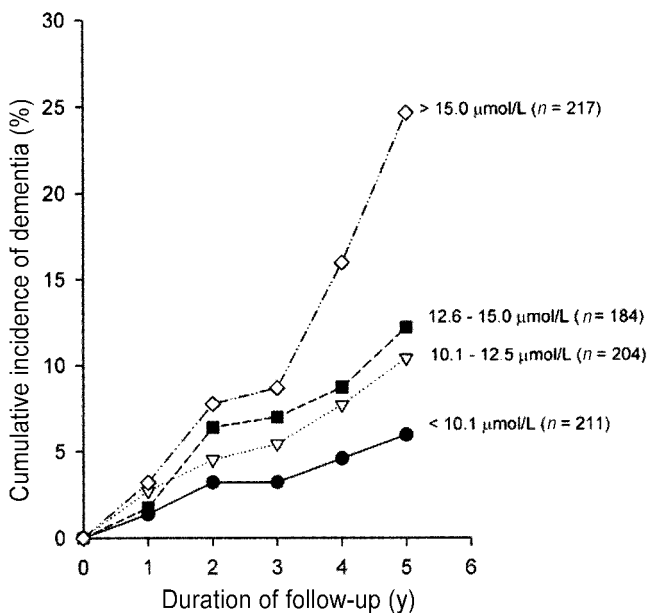


FIGURE 2. Crude cumulative incidence of dementia across quartiles of plasma total homocysteine. Data are for 816 subjects; the number of incident dementia cases was 112 (from the top to the bottom quartiles: $n = 55, 21, 23,$ and 13).

and caffeine intakes, serum cholesterol, serum thyrotropin, plasma pyridoxal-5'-phosphate, and presence of a common methylenetetrahydrofolate reductase genetic polymorphism associated with hyperhomocysteinemia (677C→T) were also

measured at baseline, but adjustment for these additional variables did not alter the results significantly (data not shown).

Effect of AD diagnostic criteria

To exclude that the association between plasma tHcy and AD resulted from inclusion of subjects who might have vascular dementia rather than AD, all analyses were repeated after the exclusion of 9 subjects with possible AD, who also had clinical or brain imaging evidence of relevant cerebrovascular disease [AD with cerebrovascular disease according to NINDS-AIREN criteria (29)]. The HR for hyperhomocysteinemia remained essentially unchanged at 2.26 (95% CI: 1.22, 4.19; $P = 0.010$). The corresponding HR for each 1-SD increase in log-transformed baseline tHcy concentrations was 1.56 (95% CI: 1.23, 1.96; $P < 0.001$).

Effect of serum folate and vitamin B-12 concentrations

In our Cox proportional hazards models, the association of plasma tHcy and dementia or AD risk was independent of B vitamin concentrations. However, even after adjustment for tHcy and all the study covariates, low folate concentrations (≤ 11.8 nmol/L) were independently related to dementia (1.87; 95% CI: 1.21, 2.89; $P = 0.005$) and AD risk (1.98; 95% CI: 1.15, 3.40; $P = 0.014$). The cumulative incidence of dementia by quartiles of folate concentrations (cutoffs were $< 8.9, 8.9-11.8, 11.9-15.2,$ and > 15.2 nmol/L) is shown in **Figure 4**. Compared with the top folate quartile, adjusted HRs for dementia were 2.22 (95% CI: 1.21, 4.05; $P = 0.010$) for the bottom quartile, 1.83 (95% CI: 1.00, 3.34; $P = 0.050$) for the lower second, and 1.16 (95%

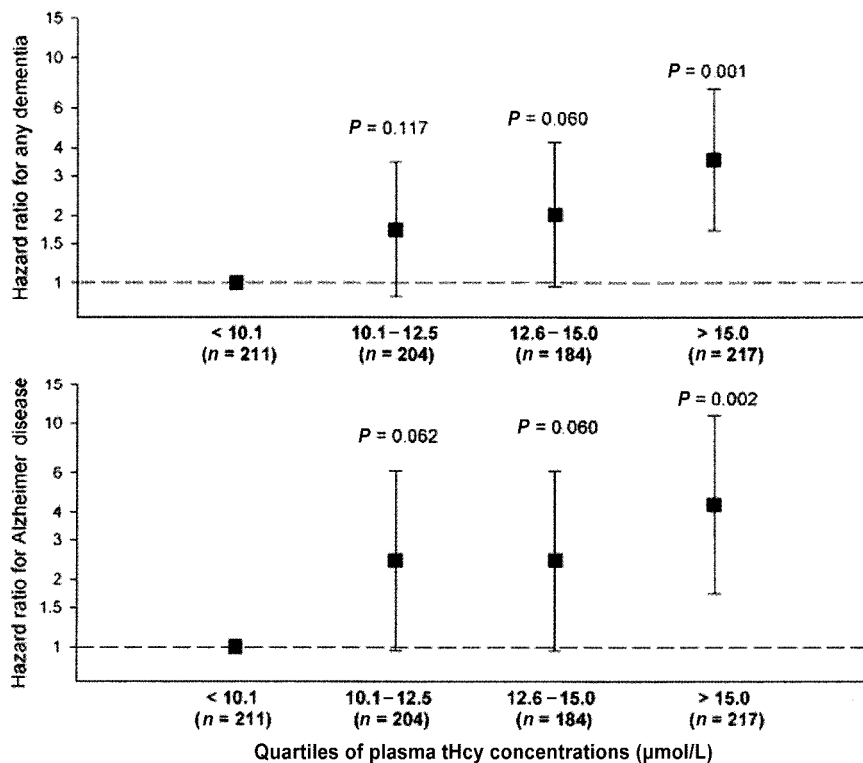


FIGURE 3. Hazard ratios and 95% CIs for any dementia and Alzheimer disease across quartiles of plasma total homocysteine. P for trend < 0.001 for any dementia and 0.002 for Alzheimer disease. Data are for 816 subjects; the number of incident dementia cases was 112 (from the top to the bottom quartiles: $n = 55, 21, 23,$ and 13) for any dementia and 70 (from the top to the bottom quartiles: $n = 31, 14, 17,$ and 8) for Alzheimer disease. The analyses were performed by using a Cox proportional hazards regression model adjusted for age, sex, education, apolipoprotein E genotype, serum creatinine, serum folate, serum vitamin B-12, and history of stroke.

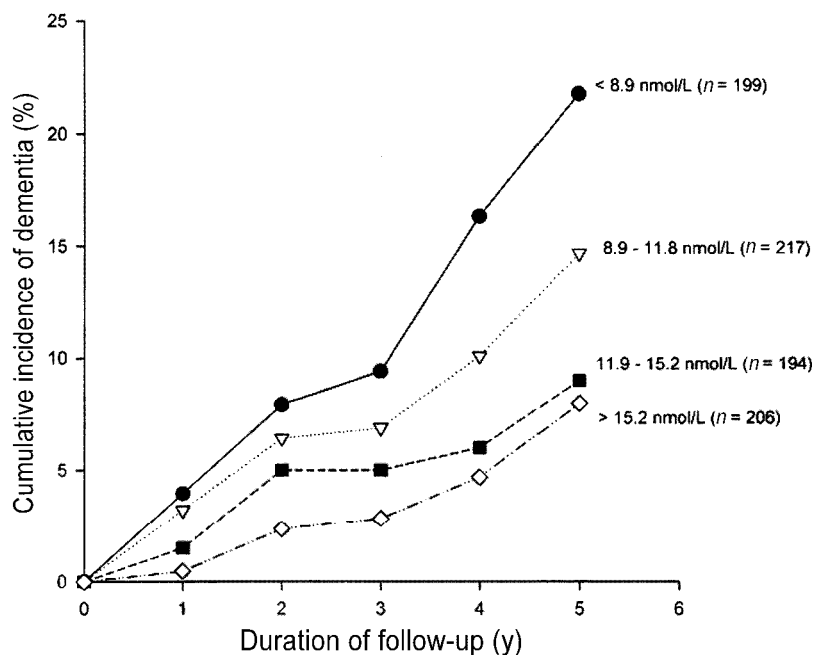


FIGURE 4. Crude cumulative incidence of dementia across quartiles of serum folate. Compared with the top folate quartile, hazard ratios and 95% CIs for dementia were 2.22 (95% CI: 1.21, 4.05; $P = 0.010$) for the bottom quartile, 1.83 (95% CI: 1.00, 3.34; $P = 0.050$) for the lower second quartile, and 1.16 (95% CI: 2.24, 0.60; $P = 0.664$) for the third quartile; P for trend = 0.004. Data are for 816 subjects; the number of incident dementia cases was 112 (from the top to the bottom quartiles: $n = 18, 18, 32,$ and 44). The analyses were performed by using a Cox proportional hazards regression model adjusted for age, sex, education, apolipoprotein E genotype, serum creatinine, plasma total homocysteine, vitamin B-12, and history of stroke.

CI: 2.24, 0.60; $P = 0.664$) for the third (P for trend = 0.004). The corresponding adjusted HRs for AD were 2.04 (95% CI: 1.02, 4.09; $P = 0.045$) for the bottom folate quartile, 1.30 (95% CI: 0.62, 2.72; $P = 0.484$) for the lower second, and 0.66 (95% CI: 0.29, 1.54; $P = 0.340$) for the third (P for trend = 0.015). By contrast, adjusted HRs relating low vitamin B-12 concentrations to risk of developing dementia (0.83; 95% CI: 0.56, 1.24; $P = 0.368$) or AD (0.66; 95% CI: 0.40, 1.09; $P = 0.103$) were not statistically significant. Results did not change when the analyses were performed with B vitamins used as log-transformed continuous variables (data not shown).

DISCUSSION

This prospective population-based study was the first to replicate previous findings from the Framingham Study (17), indicating that hyperhomocysteinemia doubles the risk of developing dementia and AD independently of several major confounders. Our results disagree with the negative findings recently reported in the WHICAP study (18). Possible explanations for this difference are the acknowledged insufficient statistical power of the WHICAP study, the rather homogeneously high tHcy concentrations of its sample—which did not permit enough variability to detect an association—and methodologic issues related to the prolonged time between blood sample collection and processing, which could have affected tHcy measurements.

Inconsistent results were also given by the only 2 studies that examined the association between homocysteine and cognitive decline at follow-up as measured with the MMSE (30, 31). These studies, however, differed in sample size and in which confounders were taken into account. Moreover, MMSE is a reliable

global screening measure of cognitive function but was not developed to estimate changes in cognitive function or to diagnose dementia (32).

The substantial evidence that tHcy is an independent vascular risk factor (5) supports the role of hyperhomocysteinemia in AD. Subjects with vascular risk factors and cerebrovascular disease have an increased risk of AD (6), and hyperhomocysteinemia has been related to cerebral macro- and microangiopathy, endothelial dysfunction, impaired nitric oxide activity, and increased oxidative stress (33-35). Moreover, as shown in cell cultures, homocysteine can directly cause brain damage through several mechanisms: increased glutamate excitotoxicity via activation of *N*-methyl-D-aspartate receptors (2), enhancement of β -amyloid peptide generation (4), impairment of DNA repair, and sensitization of neurons to amyloid toxicity (3).


On the basis of cross-sectional observations, some authors have suggested that elevated plasma tHcy concentrations are not a causative factor in dementia and AD but are only a marker for concomitant vascular disease, independently of cognitive status (36, 37). Results from other cross-sectional investigations (9, 12, 38), as well as those from the present investigation and the Framingham Study (17), argue against this interpretation, but only intervention trials can give the ultimate proof of a causal relation between hyperhomocysteinemia and AD.

In contrast with both the Framingham (17) and WHICAP (18) studies, we also found that, independent of homocysteine and other confounders (including vitamin B-12), low serum folate is associated with an increased risk of incident dementia and AD. Mandatory folate fortification of food might partially explain the negative results of the US studies, whereas in Italy, where folate fortification is not practiced, relative folate deficiency may be

endemic among the elderly population. Nondemented patients with poor cognitive performance and AD patients often exhibit poor folate status (reviewed in 8), but only one study specifically examined B vitamins in relation to incident dementia. In a selected sample of nondemented Swedish elderly participants in the Kungsholmen Study, low serum folate and vitamin B-12 were predictive of AD at 3 y of follow-up (39). The sample, however, was small (370 subjects), and a clear association was detected only when both vitamins were taken into account.

Biologic explanatory mechanisms relating folate deficiency to dementia include impaired methylation reactions in the central nervous system, with a consequent insufficient supply of methyl groups, which are required for the synthesis of myelin, neurotransmitters, membrane phospholipids, and DNA (8). However, because of the study design and the relatively short follow-up time, we cannot definitely establish whether the independent association between low folate and dementia risk indicates an actual effect of folate status on cognitive function or, on the contrary, that subtle functional alterations may affect the dietary intake of folate in the early preclinical stages of dementia.

This study has several strengths. The CSBA is a prospective population study specifically designed for the diagnosis of dementia and AD, follow-up was relatively long, and complete data on laboratory and cognitive status at follow-up was available for 87% of the cohort that was dementia-free at baseline.

This study also had several limitations. First, the lack of standardized cutoffs for hyperhomocysteinemia forced us to calculate our own reference intervals using a subsample of presumed healthy CSBA participants (15) that was not large enough to establish reliable age- and sex-specific limits. However, both age and sex were taken into account into multivariable analyses, and results for hyperhomocysteinemia were similar to those obtained when considering tHcy as a continuous variable. Therefore, it is unlikely that our results were biased by our choice of cutoff for hyperhomocysteinemia. Other limitations include the single time measurement of tHcy and the evaluation of B vitamin status as serum concentrations. Moreover, although B vitamin supplementation is recognized as the most effective means for lowering homocysteine concentrations (40), there have been no prospective trials of the effect of vitamin supplementation on the incidence of dementia. Therefore, our findings cannot be used as a basis for treatment recommendations. However, they emphasize the need for clinical trials in humans to verify whether interventions that restore folate status and reduce plasma tHcy concentrations can reduce the risk of dementia and AD in the Italian population. 

Research was facilitated by collaboration with Conselice Municipal Administration and the Local Health Unit of Ravenna, Emilia Romagna region. We are especially indebted to Edoardo Dalmonte (head physician of the Division of Geriatric Medicine at the Ravenna Hospital), Marisa Bianchin (general manager of the Lugo Medical District), and the general practitioners of Conselice for their invaluable help in organizing the study. We also express our sincerest gratitude to the population of Conselice for their enthusiasm and participation in the study.

GR was the main contributor to the study design. PF and FM contributed substantially to the data collection, data analysis, and preparation of the manuscript. MM, LS, and NB contributed to the data collection and interpretation. EP and FL contributed expert methodologic advice and edited the manuscript. None of the authors had a financial or personal interest in any organization sponsoring the research or advisory board affiliations.

REFERENCES

1. Leifer B. Early diagnosis of Alzheimer's disease: clinical and economic benefits. *J Am Geriatr Soc* 2003;51:S281-8.
2. Lipton SA, Kim WK, Choi YB, et al. Neurotoxicity associated with dual actions of homocysteine at the *N*-methyl-D-aspartate receptor. *Proc Natl Acad Sci U S A* 1997;94:5923-8.
3. Krugman II, Kumaravel TS, Lohani A, et al. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *J Neurosci* 2002;22:1752-62.
4. Sai X, Kawamura Y, Kokame K, Yamaguchi H, et al. Endoplasmic reticulum stress-inducible protein, Herp, enhances presenilin-mediated generation of amyloid beta-protein. *J Biol Chem* 2002;277:12915-20.
5. The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke. A meta-analysis. *JAMA* 2002;288:2015-22.
6. Kalaria R. Similarities between Alzheimer's disease and vascular dementia. *J Neurol Sci* 2002;203-204:29-34.
7. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 2004;50:3-32.
8. Calvaresi E, Bryan J. B vitamins, cognition, and aging: a review. *J Gerontol Psychol Sci Soc Sci* 2001;56:P327-39.
9. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer Disease. *Arch Neurol* 1998;55:1449-55.
10. McCaddon A, Davies G, Hudson P, Tandy S, Cattell H. Total serum homocysteine in senile dementia of Alzheimer type. *Int J Geriatr Psychiatry* 1998;13:235-9.
11. Lehman 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.
12. Quadri P, Fragiaco C, Pezzati R, et al. Homocysteine, folate, and vitamin B12 in mild cognitive impairment, Alzheimer disease, and vascular dementia. *Am J Clin Nutr* 2004;80:114-22.
13. Prins ND, den Heijer T, Hofman A, et al. Homocysteine and cognitive function in the elderly. The Rotterdam Scan Study. *Neurology* 2002;1375-80.
14. Budge M, Johnston C, Hogervorst E, et al. Plasma total homocysteine and cognitive performance in a volunteer elderly population. *Ann N Y Acad Sci* 2000;903:407-10.
15. Ravaglia G, Forti P, Maioli F, et al. Homocysteine and cognitive function in healthy elderly community dwellers in Italy. *Am J Clin Nutr* 2003;77:668-73.
16. Riggs KM, Spiro A, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 1996;63:306-14.
17. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476-83.
18. Luchsinger JA, Tang MX, Shea S, Miller J, Green R, Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. *Neurology* 2004;62:1972-6.
19. Ravaglia G, Forti P, Maioli F, et al. Education, occupation, and prevalence of dementia: findings from the Conselice study. *Dement Geriatr Cogn Disord* 2002;14:90-100.
20. Ravaglia G, Forti P, Maioli F, et al. Conselice study: a population based survey of brain aging in a municipality of the Emilia Romagna region (A.U.S.L. Ravenna). Design and methods *Arch Gerontol Geriatr* 2001;33(suppl):313-24.
21. Valente C, Maione P, Lippi A, et al. Validation of the Mini-Mental State Examination (MMSE) as a screening instrument for dementia in an Italian population. *Giorn Gerontol* 1992;40:161-5.
22. Magni E, Binetti G, Bianchetti A, Rozzini R, Trabucchi M. Mini-Mental State Examination: a normative study in an Italian elderly population. *Eur J Neurol* 1996;3:1-5.
23. Carlesimo GA, Caltagirone C, Gainotti G. The Mental Deterioration Battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. The Group for the Standardization of the Mental Deterioration Battery. *Eur Neurol* 1996;36:378-84.
24. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association, 1994.
25. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under



- the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-44.
26. Licastro F, Pedrini S, Ferri C, et al. Gene polymorphism affecting α 1-antichymotrypsin and interleukin-1 plasma levels increases Alzheimer's disease risk. *Ann Neurol* 2000;48:388-91.
 27. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993;270:2693-8.
 28. Herrmann W, Quast S, Ullrich M, Schultze H, Bodis M, Geisel J. Hyperhomocysteinemia in high-aged subjects: relation of B-vitamins, folic acid, renal function and the methylenetetrahydrofolate reductase mutation. *Atherosclerosis* 1999;144:91-100.
 29. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology* 1993;43:250-60.
 30. McCaddon A, Hudson P, Davies G, Hughes A, Williams JHH, Wilkinson C. Homocysteine and cognitive decline in healthy elderly. *Dement Geriatr Cogn Disord* 2001;12:309-13.
 31. Kalmijn S, Launer LJ, Lindemans J, Bots ML, Hofman A, Breteler MMB. Total homocysteine and cognitive decline in a community-based sample of elderly subjects. The Rotterdam Study. *Am J Epidemiol* 1999; 150:283-9.
 32. Tombaugh TN, McIntyre NJ. The Mini-Mental State Examination: a comprehensive review. *J Am Geriatr Soc* 1992;40:922-35.
 33. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;338:1042-50.
 34. Fassbender K, Mielke O, Bertsch T, Nafe B, Froschen S, Hennerici M. Homocysteine in cerebral macroangiopathy and microangiopathy. *Lancet* 1999;353:1586-7.
 35. Hajjar K. Homocysteine: a sulph'rous fire. *J Clin Invest* 2001;107: 663-4.
 36. Miller JW, Green R, Mungas DM, Reed BR, Jagust WJ. Homocysteine, vitamin B6, and vascular disease in AD patients. *Neurology* 2002;58: 1471-5.
 37. Borroni B, Agosti C, Panzali AF, Di Luca M, Padovani A. Homocysteine, vitamin B6, and vascular disease in patients with AD. *Neurology* 2002;59:1475-6.
 38. Storey SG, Suryadevara V, Aronow WS, Ahn C. Association of plasma homocysteine in elderly persons with atherosclerotic vascular disease and dementia, atherosclerotic vascular disease without dementia, dementia without atherosclerotic vascular disease, and no dementia or atherosclerotic vascular disease. *J Gerontol Med Sci* 2003;58:M1135-6.
 39. Wang HX, Wahlin A, Basun H, Fastbom J, Winblad B, Fratiglioni L. Vitamin B12 and folate in relation to the development of Alzheimer's disease. *Neurology* 2001;56:1188-94.
 40. Malinow MR, Bostom AG, Krauss RM. Homocyst(e)ine, diet and cardiovascular diseases. A statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 1999; 99:178-82.