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Plasma Homocysteine and Risk of Mild Cognitive Impairment

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Key Words
Homocysteine · Dementia · Mild cognitive impairment

Abstract
Background and Objective: There are conflicting data relating homocysteine levels to the risk of Alzheimer's disease (AD). We sought to explore whether fasting plasma homocysteine is associated with the risk of mild cognitive impairment (MCI), an intermediate stage to dementia. ***Methods:*** Fasting levels of plasma homocysteine were obtained from 678 elderly subjects chosen at random from a cohort of Medicare recipients. There were longitudinal data in 516 subjects without MCI or dementia at baseline who were followed for 2,705 person-years. The relation of plasma homocysteine with prevalent and incident all-cause MCI, amnestic MCI and non-amnestic MCI was assessed using logistic and Cox proportional hazards regression analyses. ***Results:*** There were 162 cases of prevalent MCI and 132 cases of incident MCI in 5.2 years of follow-up. There was no association between plasma homocysteine and prevalence of MCI or amnestic or non-amnestic MCI in the cross-sectional analyses. There was no association between higher homocysteine levels and a lower risk of all-cause MCI. Consistent with the cross-sectional analyses, there was no specific association with the amnestic or non-amnestic subtype of MCI in crude or adjusted models. ***Conclusion:*** Plasma homocysteine levels measured at baseline were not related to MCI or its subtypes in an elderly multiethnic cohort.

Introduction

The prevalence of Alzheimer's disease (AD) is expected to quadruple by the year 2047 [1]. Delaying the onset by a few years would decrease its prevalence and public health burden [1]. There are no known cures or preventive measures, but there is growing evidence that modifiable vascular risk factors may have an important role in its etiology [2]. A potentially important risk factor for heart disease and stroke is hyperhomocysteinemia [3–6]. Homocysteine is converted by folate, vitamin B_{12} and B_6 to methionine and cysteine. Homocysteine levels in blood increase with age and with diminishing renal function, but are largely determined by dietary intake and levels of vitamins B_{12}, B_6, and folate [7], but increase with age and with diminishing renal function. Thus, homocysteine levels can be modified through dietary interventions.

Previous studies relating homocysteine levels with the risk of dementia were inconsistent [8–13]. While some longitudinal data show an association between hyperho-

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mocysteinemia and a higher risk of AD, other studies reported inverse or no associations [8–13]. We previously explored the associations of homocysteine levels with risk of AD and amyloid beta (Aβ) protein and observed a relation between homocysteine levels and Aβ40 but not Aβ42 or AD [14, 15].

As a transitional stage between normal cognition and dementia and a target for early treatment and prevention, mild cognitive impairment (MCI) has attracted increasing interest over the past years. Studies using the criteria by Petersen et al. [16, 17] for diagnosing MCI in clinical and epidemiological settings report an incidence rate of 9.9/1,000 person-years for MCI among non-demented elderly [18], and an annual conversion rate of 10–12% to AD in subjects with MCI, particularly amnestic MCI, in contrast to a conversion rate of 1–2% in the normal elderly population [17]. Recent data suggest that non-amnestic MCI progresses to dementia at a markedly lower rate than amnestic MCI [19], and that non-amnestic MCI is probably more representative of vascular cognitive impairment than amnestic MCI [20].

The objective of the present study was to determine whether or not homocysteine is associated with the risk of MCI or its amnestic or non-amnestic subtypes. Clarification of the association between homocysteine levels and cognitive impairment can help understand the etiology of cognitive decline, can help identify persons at risk which could benefit from dietary intervention, and can help design strategies for prevention and treatment.

Methods

Subjects and Setting

Participants were enrolled in a longitudinal cohort study by a random sampling of Medicare recipients 65 years or older residing in northern Manhattan (Washington Heights, Hamilton Heights, Inwood). The sampling procedures have been described elsewhere [21]. Each participant underwent an in-person interview of general health and function at the time of study entry followed by a standard assessment, including medical history, physical and neurological examination as well as a neuropsychological battery [22]. Baseline data were collected from 1992 through 1994. Follow-up data were collected during evaluations at sequential intervals of approximately 18 months, performed from 1992 to 2006. This study was approved by the institutional review board of the Columbia-Presbyterian Medical Center.

Plasma homocysteine was measured in a subsample of the cohort chosen at random [15]. The sample for this study comprised those participants who were without MCI or dementia at baseline, who had measures of plasma homocysteine levels, and who had complete information to ascertain MCI following the Petersen criteria [16, 17]. Of the 1,772 participants in whom a full neuropsychological examination was attempted, 371 (20.9%) were excluded due to prevalent dementia, and 723 (40.8%) were not part of the homocysteine subsample. Thus, the final analytic sample included 678 individuals. Compared to the original 1,772 participants, the final sample was younger (77.3 vs. 78.1 years; $p = 0.01$), had similar proportions of women (70.3 vs. 69.2%; $p = 0.64$); non-Hispanic Whites (18.7 vs. 21.0%; $p = 0.25$), African-Americans (31.1 vs. 34.9%; $p = 0.09$), diabetes (16.7 vs. 16.7, $p = 0.1$), heart disease (14.9 vs. 17.9%, $p = 0.1$) and hypertension (51.3 vs. 50.5%, $p = 0.7$), and had a higher proportion of Hispanics (50.2 vs. 44.0%; $p = 0.01$).

Clinical Assessments

Data were available from medical, neurological, and neuropsychological evaluations [22]. All participants underwent a standardized neuropsychological test battery examining multiple domains at baseline and subsequent assessments using the Mini-Mental State Examination, the Boston Naming Test, the Controlled Word Association Test, category naming, the Complex Ideational Material and Phrase Repetition subtests from the Boston Diagnostic Aphasia Evaluation, the WAIS-R Similarities subtest, the Mattis Dementia Rating Scale, the Rosen Drawing Test, the Benton Visual Retention Test, the multiple choice version of the Benton Visual Retention Test and the Selective Reminding Test [22].

Diagnosis of Dementia and MCI

Dementia was diagnosed by consensus of neurologists, psychiatrists and neuropsychologists based on DSM-IV criteria [23]. Consistent with standard criteria [17] for all subtypes of MCI, those considered for MCI were required to have: (1) memory complaint; (2) objective impairment in at least one cognitive domain based on the average of the scores on the neuropsychological measures within that domain and a 1.5-SD cutoff using normative corrections for age, years of education, ethnicity, and sex; (3) essentially preserved activities of daily living, and (4) no dementia. Participants with MCI were stratified into those with: (1) isolated impairment in memory or impairment in memory and one or more other cognitive domains ('amnestic MCI') or (2) no impairment in memory but impairment in two or more other cognitive domains ('non-amnestic MCI'), as described in detail previously [24].

Plasma Homocysteine Levels

Blood was drawn at baseline under fasting conditions. It was drawn into EDTA tubes, and centrifuged, separated into plasma aliquots, and stored at –70° C within 2 h of collection. Homocysteine levels were measured from plasma using high-performance liquid chromatography with fluorescence detection [25].

Other Covariates

APOE genotyping was determined using the method of Hixson and Vernier [26]. Participants were classified as positive for the APOE-ε4 allele genotype if they had one or two ε4 alleles. Plasma folate and vitamin B_{12} were determined by radioassay (Simultrac, ICN Pharmaceuticals, Costa Mesa, Calif., USA). Plasma pyridoxal-5′-phosphate, an indicator of vitamin B_6 status, was determined by radioenzymatic assay (ALPCO, Wyndham, N.H., USA). Creatinine was measured by spectrophotometric assay (Sigma, St. Louis, Mo., USA). Stroke was defined according to the WHO criteria [27]. The presence of stroke was ascertained from

Table 1. Characteristics of the study sample in cross-sectional analyses

Women	477 (70.3%)
Age, years	77.3 ± 5.8
Education, years	8.3 ± 4.7
Ethnic group[a]	
White/non-Hispanic	126 (18.7%)
Black/non-Hispanic	210 (31.1%)
Hispanic	340 (50.2%)
APOE genotype 4/– or 4/4	181 (26.7%)
Homocysteine, μmol/l	16.8 ± 7.9
Creatinine, mg/dl	1.1 ± 1.1
Vitamin B_{12}, pg/ml	387.1 ± 303.6
Folate, ng/ml	8.1 ± 5.9
Stroke	63 (9.3%)
Diabetes	114 (16.7%)
Heart disease	95 (14.9%)
Current smoking	100 (14.7%)
Hypertension	348 (51.3%)
MCI	162 (23.9%)

[a] Classified by self-report using the format of the 1990 US census.

an interview with participants and their informants. Persons with stroke were confirmed through their medical records, 85% of which included results of brain imaging. The remainder was confirmed by direct examination.

Statistical Methods

First we evaluated the demographic and clinical characteristics of the study sample at baseline. The distributions of homocysteine, cysteine, vitamin B_{12} and folate were skewed and were transformed before further analyses using natural logarithm to achieve normal distributions. Then levels of homocysteine, folate and vitamin B_{12} were compared between persons with and without MCI at baseline using ANOVA. Logistic regression analyses were used to relate homocysteine levels to prevalent all-cause MCI, amnestic MCI and non-amnestic MCI. Each outcome was examined in a separate model. Cox proportional hazards regression was used to relate homocysteine levels to incident MCI and MCI subtypes, the time-to-event variable in these models was from age at baseline to age at onset of MCI. Among individuals who did not develop MCI, those who developed dementia were censored at the time of dementia diagnosis, and those who did not develop dementia, who died, or who were lost to follow-up owing to relocation before development of MCI were censored at the time of their last evaluation. We first performed crude models, subsequently we adjusted all models for age, gender, ethnic group and APOE-ε4, and then in addition for creatinine. Homocysteine levels were explored as a continuous variable, in tertiles, and as a dichotomized variable using the acknowledged level of 14 μmol/l as the cutoff. Data analysis was performed using SPSS version 15.0 software (SPSS Inc., Chicago, Ill., USA) and SAS 9.1 for Windows (Cary, N.C., USA).

Results

Characteristics of the study sample are shown in table 1. The mean age of the sample of 678 subjects was 77.4 ± 5.8 years, and 70.4% were women. There were 162 cases of prevalent MCI and 132 cases of incident MCI in ~5.2 years of follow-up. This corresponds to a follow-up time of 2,705 person-years. Out of the 132 persons who developed MCI during follow-up, 78 persons (59.1%) developed MCI at the first follow-up interval, 52 persons (39.4%) at the second follow-up interval, and 2 persons (1.5%) at the third follow-up interval. Of the 384 persons who remained free of MCI during follow-up, 81 (21.1%) were censored at the first follow-up, 101 (26.3%) at the second follow-up, 71 (18.5%) at the third follow-up, 24 (6.3%) at the fourth follow-up, 41 (10.7%) at the fifth follow-up and 66 at the sixth follow-up (17.2%). Levels of homocysteine, vitamin B_{12} or folate did not significantly differ between persons with and without MCI at baseline (table 2). Homocysteine levels at baseline did also not differ between persons who remained free of MCI during follow-up (mean ± SD: 17.32 ± 9.1 μmol/l) or persons who developed MCI during the course of the study (mean ± SD: 16.03 ± 6.0 μmol/l).

The mean age at onset of MCI was 81.2 ± 5.9 years. There was no association between plasma homocysteine levels and prevalence of MCI or amnestic or non-amnestic subtype of MCI in the cross-sectional analyses (table 3). In the longitudinal analyses, there was a trend towards an association between higher homocysteine levels and a lower risk of all-cause MCI in crude models, but this risk was appreciably attenuated with adjustment for ethnic group and APOE-ε4 genotype (table 4). There was also no specific association with the amnestic or non-amnestic subtype of MCI in the longitudinal analyses. Additional adjustment for creatinine, or using time to event or last evaluation as the time variable did not change these relations.

Discussion

In this multiethnic urban cohort, higher plasma levels of homocysteine measured at baseline were not associated with all-cause MCI, amnestic MCI or non-amnestic MCI in cross-sectional or longitudinal analyses.

The mechanisms through which homocysteine could affect cognitive function remain controversial. Higher homocysteine levels could lead to cognitive impairment through cerebrovascular disease [13, 28, 29] or increased

Table 2. Comparison of transformed[a] homocysteine, vitamin B_{12} and folate levels across persons with normal cognition, all-cause MCI, amnestic MCI or non-amnestic MCI at baseline

	Normal cognition (n = 516)	All-cause MCI (n = 162)	Amnestic MCI (n = 65)	Non-amnestic MCI (n = 97)
Homocysteine levels				
Mean ± SD	2.77 ± 0.36	2.72 ± 0.34	2.69 ± 0.39	2.75 ± 0.33
<14 μmol/l	193 (37.4%)	70 (43.2)	28 (43.1)	42 (43.4)
≥14 μmol/l	323 (62.6%)	92 (56.8)	37 (56.9)	55 (56.7)
Vitamin B_{12}, pg/ml	5.72 ± 0.72	5.77 ± 0.73	5.82 ± 0.60	5.74 ± 0.80
Folate, ng/ml	1.89 ± 0.57	1.96 ± 0.64	2.02 ± 0.57	1.92 ± 0.67

[a] Levels were transformed using natural logarithm.

cortical or hippocampal atrophy [30]. It is also possible that vitamins involved in homocysteine metabolism such as folic acid, vitamin B_6 and vitamin B_{12} [31], confound relations between homocysteine and cognition or vice versa [32] or that homocysteine itself has neurotoxic and excitotoxic properties. However, the evidence for neurotoxic effects comes largely from in vitro studies [33, 34], and several studies suggested that elevated homocysteine levels in persons with cognitive impairment or dementia are not a cause of but reflect concomitant vascular disease [35].

Previous studies relating homocysteine levels with the risk of dementia or MCI were inconsistent. Some cross-sectional or longitudinal studies reported associations between elevated homocysteine levels and an increased risk of cognitive impairment [13, 36–39]. The Framingham Study reported a twofold increased risk of AD for individuals in the highest quartile of homocysteine levels after adjustment for age [13]. The Sacramento Area Latino Study on Aging reported an association between higher homocysteine levels and a combined outcome of cognitive impairment no dementia and dementia [10]. In other studies, elevated serum concentrations of homocysteine were associated with cognitive impairment in elderly persons but not with an increased rate of cognitive decline [11] indicating that high serum concentrations of homocysteine may be a consequence but not cause of the disease. Finally, in several studies, homocysteine levels were not associated with cognitive impairment [8, 9, 40].

MCI, an intermediate stage between normal cognition and dementia [41], is increasingly studied as a cognitive outcome in research and clinical practice. MCI has been characterized into subtypes [42, 43]. Amnestic MCI is thought to be more specific to AD, while non-amnestic MCI seems to be related to other causes such as cerebrovascular disease [42]. This notion is supported by recent work in our cohort that has shown that risk factors for AD are also risk factors for amnestic MCI, such as diabetes [44], while risk factors for vascular dementia, such as hypertension [20], and also diabetes [45] are also risk factors for non-amnestic MCI. We had previously found that

Table 3. OR and 95% CI relating plasma homocysteine levels with all-cause MCI, amnestic MCI and non-amnestic MCI in cross-sectional analyses

	Model 1 OR (95% CI)	Model 2 OR (95% CI)
All-cause MCI		
1st tertile	reference	reference
2nd tertile	0.9 (0.55–1.29)	0.8 (0.54–1.28)
3rd tertile	0.8 (0.50–1.18)	0.7 (0.47–1.14)
p for trend	0.2	0.2
per unit increase (μmol/l)	0.8 (0.44–1.25)	0.7 (0.41–1.19)
Amnestic MCI		
1st tertile	reference	reference
2nd tertile	0.9 (0.48–1.61)	0.9 (0.48–1.60)
3rd tertile	0.5 (0.28–1.06)	0.5 (0.27–1.07)
p for trend	0.09	0.08
per unit increase (μmol/l)	0.6 (0.27–1.25)	0.6 (0.25–1.23)
Non-amnestic MCI		
1st tertile	reference	reference
2nd tertile	0.8 (0.48–1.40)	0.8 (0.46–1.37)
3rd tertile	0.9 (0.57–1.58)	0.9 (0.51–1.49)
p for trend	0.8	0.6
per unit increase (μmol/l)	0.9 (0.47–1.65)	0.8 (0.43–1.58)

Model 1 is a crude model. Model 2 is adjusted for age, gender, ethnic group, APOE-ε4.

Table 4. Hazard ratios (HR) and 95% CI relating plasma homocysteine levels and the risk of incident MCI

MCI subtype	Number of incidents MCI (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
All-cause MCI			
1st tertile	44 (25.6)	reference	reference
2nd tertile	49 (28.3)	1.0 (0.64–1.44)	1.0 (0.68–1.56)
3rd tertile	39 (22.8)	0.7 (0.46–1.08)	1.0 (0.63–1.54)
p for trend		0.1	0.9
per unit increase (μmol/l)	132 (25.6)	0.6 (0.35–0.92)*	0.9 (0.53–1.47)
Amnestic MCI			
1st tertile	15 (10.5)	reference	reference
2nd tertile	17 (12.1)	1.0 (0.50–2.00)	1.1 (0.53–2.22)
3rd tertile	17 (11.4)	0.9 (0.42–1.70)	1.3 (0.60–2.58)
p for trend		0.6	0.5
per unit increase (μmol/l)	49 (11.3)	0.6 (0.29–1.32)	1.0 (0.44–2.12)
Non-amnestic MCI			
1st tertile	29 (18.5)	reference	reference
2nd tertile	32 (20.5)	1.0 (0.57–1.57)	1.1 (0.63–1.76)
3rd tertile	22 (14.3)	0.6 (0.37–1.11)	0.9 (0.51–1.59)
p for trend		0.1	0.7
per unit increase (μmol/l)	83 (17.8)	0.5 (0.29–1.02)	0.9 (0.44–1.67)

Cox proportional hazards model, with age at onset as time variable, as described in the text. Model 1 is a crude model. Model 2 is adjusted for age, gender, ethnic group, APOE-ε4.

* Significant at a 0.05 level.

homocysteine was not associated with AD in longitudinal analyses, and others reported an association with vascular disease but not AD pathology [46]. Thus, we would have expected an association with non-amnestic MCI, and no association with amnestic MCI. However, in neither cross-sectional nor longitudinal analyses adjusting for age, sex and other potential confounders were homocysteine levels associated with prevalence or risk of MCI. Results from trials assessing the impact of homocysteine-lowering treatment on cognition have been inconclusive [47–49]. The most definitive data to date on the relation between homocysteine and cognition comes from a 2-year, double-blind, placebo-controlled, randomized clinical trial of homocysteine-lowering treatment with B_{12}, B_6 and folate supplements in 276 elderly participant with high plasma homocysteine by McMahon et al. [47]. This trial demonstrated a lowering of plasma homocysteine in the treatment group. However, this was not accompanied by better cognitive performance. The results of our study are in agreement with those showing no association and with the results of McMahon et al.

The third tertile of homocysteine in our analyses was related to a lower risk of MCI which was not statistically significant. This could be interpreted as deviating markedly from previous literature and could be dismissed as caused by selection bias or variability in a small sample. However, there is increasing evidence that the interaction among the vitamins that determine homocysteine levels is complex and that higher intake of these vitamins could result in worse cognition. A cross-sectional analysis of the National Health and Nutrition Examination Survey data showed that in elderly people with low serum B_{12}, high folate levels were related to cognitive impairment [50]. A longitudinal analysis in the Chicago Health and Aging Project showed that higher intake of folate was related to cognitive decline in the elderly [51]. An analysis from our cohort showed that higher folate intake was related to lower risk of AD that became apparent after controlling for B_{12} intake. Lastly, post-hoc analyses in the trial by McMahon et al. [47] suggested that persons in the vitamin supplementation group had worse cognitive performance despite a decrease in homocysteine levels. More studies are needed examining how the complex interactions among the vitamins that determine homocysteine levels affect cognition.

We must consider alternative explanations for our findings. It is possible that our sample was too homogeneous in homocysteine levels not permitting enough variability to detect a harmful association. The proportion of persons with high homocysteine levels and vascular disease in the population of Northern Manhattan is higher than in other populations in which associations between high homocysteine and a higher dementia risk have been reported [15, 52]. It is possible that most of our sample may have been at a high risk of dementia given relatively high homocysteine levels. However, we conducted secondary longitudinal analyses classifying homocysteine using the 14 μmol/l cutoff point and found no association (all-cause MCI: HR 0.8; 95% CI 0.54–1.10; amnestic MCI: 0.68, 95% CI 0.38–1.20; non-amnestic MCI: HR 0.84, 95% CI 0.54–1.32). Another possibility is that homocysteine levels are related to cognitive impairment in younger individuals but not the older sample in our study. Our sample was older than 65 years with a mean age of 77.5 years. It is possible that individuals with adverse outcomes related to homocysteine levels did not survive to inclusion in our study. It is also possible that in older age the brain is less vulnerable to the effects of homocysteine levels than in middle age, or that the follow-up period in this study was too short to detect a harmful effect in this elderly population. Another possibility is that we did not have enough power to find an association in a relatively small sample. At a 0.05 α level, we had 80% power to detect a relative risk of 2.0. However, the effects estimates in all tertiles in models adjusted for age and sex, particularly in the smaller longitudinal sample, were close to 1, suggesting that lack of power is not an explanation for the lack of significant results.

Limitations include that we used only one measurement of homocysteine levels, which could have led to measurement error and an underestimation of the association between homocysteine and cognitive impairment. Due to lack of repeat homocysteine measurement during follow-up we could not investigate how changes in homocysteine levels over time affect the risk to develop cognitive impairment. However, repeat measurements after 6–18 months in the elderly show good reproducibility of baseline levels with non-significant intraindividual variations of as little as 0.85–1.2 μmol/l, suggesting that such study likely would have yielded similar results [53, 54]. An important strength of our study is that it is a prospective cohort study especially designed for the diagnosis of cognitive impairment and dementia.

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