Plasma Homocysteine Is Associated with the Risk of Mild Cognitive Impairment in an Elderly Korean Population

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Abstract

Elderly individuals with mild cognitive impairment (MCI) are at high risk for developing dementia, including Alzheimer’s disease. Previous studies have proposed that elevated plasma homocysteine might be a risk factor for dementia. However, the impact of plasma homocysteine on MCI remains controversial. We investigated the relation between hyperhomocysteinemia and the risk of MCI in an elderly Korean population. A total of 1215 elderly subjects (aged 60–85 y) were selected from the Ansan Geriatric study to participate in this study. MCI was diagnosed on the basis of the Mayo Clinic criteria. Mean plasma homocysteine concentrations were higher in elderly subjects with MCI than in normal elderly subjects (17.6 ± 7.4 vs. 15.7 ± 4.8 μmol/L; P < 0.001). Subjects with hyperhomocysteinemia (>15 μmol/L) also had a higher prevalence of MCI. The unadjusted OR for MCI was greater in subjects with hyperhomocysteinemia than in normal subjects and it increased according to the degree of hyperhomocysteinemia (OR = 1.39; 95% CI = 1.09–1.79 vs. OR = 2.61; 95% CI = 1.22–5.61). These trends did not differ after adjustment for age, sex, and other putative risk factors for cognitive dysfunction (OR = 1.40; 95% CI = 1.07–1.83 vs. OR = 2.40; 95% CI = 1.08–5.31). In conclusion, hyperhomocysteinemia may be an independent risk factor for MCI in elderly Koreans. A causal relationship between plasma homocysteine levels and cognitive impairment should be evaluated in a follow-up study of elderly Korean subjects. J. Nutr. 137: 2093–2097, 2007.

Introduction

Dementia has recently become the focus of substantial research interest, given the increased number of elderly people in developed countries. Mild cognitive impairment (MCI) refers to the transitional stage between normal aging and dementia (1). In this regard, individuals with MCI are at high risk for developing dementia (2). Several studies have reported that patients with MCI develop Alzheimer’s disease at a rate of 10–15%/y, whereas the rate of incidence for healthy subjects is only 1–2% annually (3).

An inverse relationship between plasma total homocysteine (tHcy) levels and cognitive function has been reported in case-control (4–6) and cross-sectional studies (7–9). Longitudinal studies have proposed that elevated plasma homocysteine may be a risk factor for Alzheimer’s disease (10,11). However, data on the association between elevated tHcy levels and MCI are inconsistent (8,9,12–15). Furthermore, information on the risk of MCI in relation to elevated tHcy levels in the Asian population is very limited.

To the best of our knowledge, only 1 study has provided information on the relationship between elevated tHcy concentration and MCI in South Korea (14), even though South Korea is considered to have the most rapidly aging population in the world. However, this previous study did not include serum folate, vitamin B-12, or the apolipoprotein E (apoE) genotype, which are putative risk factors for cognitive decline, including Alzheimer’s disease, in the statistical analysis (16). Furthermore, the sample in that study was medium sized, with 409 controls and 102 cases with MCI.

In this study, using a large, stratified, and randomized sample from the population-based Ansan Geriatric (AGE) cohort study, we investigated the relationship between elevated plasma tHcy levels and the risk of MCI in an elderly Korean population.

Subjects and Methods

Study design and sampling population. Participants were randomly selected from the AGE study. The AGE study is an ongoing, prospective,
population-based epidemiological study that was established in May of 2002. The sampling protocol and design of the AGE study for a baseline investigation have been well described previously (17). From the database (2676 subjects aged ≥60 y), for a baseline investigation, we constructed a first-wave sample from the AGE cohort study, as described previously (18). Briefly, all subjects received a telephone call and a letter at least 3 times inviting them to attend a comprehensive health screening at the Geriatric Health Clinic and Research Institute, Korea University Hospital, Ansan, Korea. Despite extensive efforts, however, a total of only 1391 subjects (395 men and 796 women) were randomly recruited between September 2004 and March 2006 and were regarded as the first-wave sample. The first-wave sample was evaluated by clinical and neuropsychological examinations and was subjected to genotyping for apoE ε4 polymorphism. In the first-wave sample, 64 subjects with no available data for neuropsychological examinations were excluded and a further 41 were excluded, because they were diagnosed with dementia at site examinations. Among the remaining 1286 subjects, 71 were excluded because they had insufficient information on health status, smoking status, serum vitamins, and apoe ε4 genotype. Therefore, a total of 1215 elderly Korean subjects (520 men and 695 women; mean age, 68.7 ± 5.4 y; range, 60–85 y) were finally eligible for this analysis.

Informed written consent for participation was obtained from each individual and the study protocol was approved by the institutional review board of the AGE study.

Cognitive function assessment. MCI was diagnosed on the basis of the Mayo Clinic criteria (3), which include the following: 1) memory complaint, preferably corroborated by an informant; 2) objective memory impairment for age; 3) largely preserved general cognition; 4) essentially normal activities of daily living; and 5) no dementia. Diagnosis was made independently by a physician on the basis of all available information. The MCI group manifested memory or executive deficits compared with a normal score on the Korean version of the Consortium to Establish a Registry for Alzheimer’s Disease Assessment Packet (19) according to age, sex, and education (score < mean – 1.5 SD) but with no impact on activities of daily living. The cut-off score of –1.5 SD below the mean was chosen to be consistent with published criteria for MCI (3). The final diagnosis was based upon a consensus meeting, attended by at least 1 neurologist, neuropsychologist, clinical neurophysiologist, geriatrician, and specialized nurse, at which all the available clinical data were considered. Symptoms of depression were determined according to the Korean version of the Geriatric Depression Scale (20). Depression was defined as a Geriatric Depression Scale score ≥18 (20).

Laboratory procedures. Baseline blood samples were taken after an overnight fast. Blood samples for plasma homocysteine measurement were collected in tubes containing EDTA and centrifuged as soon as possible. The plasma was then separated from the cells after centrifugation, placed on ice, and kept in a refrigerator (−4°C) until analysis for homocysteine. Fasting plasma tHcy concentrations were measured with the automated ADVIA Centaur immunoassay (Bayer Healthcare) by a direct chemiluminescence method (21). We classified subjects into 4 groups in relation to plasma tHcy. We defined tHcy as severe hyperhomocysteinemia for concentrations of >100 μmol/L, intermediate for concentrations of >30 and ≤100 μmol/L, moderate for concentrations of >15 and ≤30 μmol/L, and the reference total plasma tHcy range as ≤15 μmol/L (7,22). Blood samples for serum vitamin measurements were collected in separate tubes and sent to a biochemical laboratory for analysis. Serum concentrations of folate and vitamin B-12 were measured using the Simul TRAC-SNB radioimmunoassay (ICN Biomedicals).

apoe genotypes. Genomic DNA was obtained from EDTA-treated blood using a QIamp DNA Blood Mini kit (Qiagen). apoe ε allele genotyping was performed by PCR. Subjects were divided into 2 groups based on the presence or absence of the apoe ε4 allele.

Other measurements. The well-trained field survey team was composed of university students majoring in nursing and experienced interviewers. To ensure uniformity, all interviewers underwent 2 d of training before the survey was initiated and then the sociodemographic data were collected from the subjects. McNemar’s test was used to assess the reliability between surveyors and showed no difference (P > 0.05) (23).

Detailed data on sociodemographic factors and personal characteristics were collected including age, sex, level of education, marital status, smoking, disease history, and dietary supplements. Marital status was categorized as married (cohabiting) or single (unmarried, widowed, divorced, or separated). Smoking behavior was classified as current smoker, ex-smoker (for at least 1 y), and nonsmoker, as previously described (24). Educational status was categorized as ≥6 y (elementary school completion), 7–12 y, and ≥13 y (college education level) in the Korean education system. Disease history was reported by subjects using a checklist that included 35 diseases and a residual category called “other diseases” and confirmed by reviewing medical records and medications (23). Information on dietary supplements was obtained from the subjects who answered the open-ended question: “Are you currently taking dietary supplements? If so, please write down or tell all the names.” In this study, a total of 272 elderly subjects reported taking dietary supplements that included Chinese herbs (as a restorative), multivitamins, minerals such as calcium and iron, glucosamine, and others.

Statistical analysis. Data were expressed as means with SD (continuous) or number and percentage (categorical). Differences between normal subjects and those with MCI were evaluated using Student’s t test or a chi-square test, as appropriate. Because of the skewed distributions of homocysteine, folate, and vitamin B-12, these variables were log-transformed before analysis. Differences among 3 groups according to the levels of plasma homocysteine were determined using general linear models (Duncan’s test of multiple comparisons) or chi-square test, as appropriate. Multivariable-adjusted logistic regression analysis was conducted to examine the OR for MCI across a range of plasma homocysteine levels. Relationships between homocysteine and folate or vitamin B-12 were evaluated using Pearson’s correlation analysis. Statistical analyses were performed with SAS software (SAS 9.1; SAS Institute). Statistical significance was defined as P < 0.05.

Results

Prevalence of MCI and study population characteristics. Of the 1215 elderly Koreans examined, 827 (68.1%) were considered to be in a normal state and 388 (31.9%) had MCI (population characteristics are presented in Table 1). Elderly individuals with MCI were older, less likely to be educated, and more likely to have depression. Plasma tHcy concentrations were higher in subjects with MCI (17.6 ± 7.4 μmol/L) than in normal subjects (15.7 ± 4.8 μmol/L; P < 0.001). However, serum folate and vitamin B-12 concentrations did not differ significantly between normal subjects and those with MCI. The prevalence of the apoe genotype also did not differ significantly between the 2 groups.

Sociodemographic and clinical characteristics according to the levels of plasma tHcy. The sociodemographic and clinical characteristics according to plasma homocysteine level are shown in Table 2. A total of 638 subjects (53.0%) had moderate and 28 (2.3%) had intermediate hyperhomocysteinemia. None of the subjects in this study had severe hyperhomocysteinemia and, therefore, subjects were categorized into 3 groups in relation to plasma tHcy: normal (tHcy ≤15 μmol/L), moderate (15 < tHcy ≤30 μmol/L), or intermediate (30 < tHcy ≤100 μmol/L). Subjects with hyperhomocysteinemia were older, more likely to be men, current or ex-smokers, and more likely to have low serum folate and vitamin B-12 concentrations.

Plasma homocysteine increased at each category of plasma homocysteine (P < 0.001) and serum folate and vitamin B-12
changed in the opposite direction (P < 0.001). As expected, subjects with hyperhomocysteinemia had higher prevalence of MCI (P = 0.009).

**OR and 95% CI for MCI according to plasma tHcy**. The overall results relating plasma tHcy level to the risk of MCI are shown in Table 3. The unadjusted OR for MCI increased with moderate hyperhomocysteinemia (OR = 1.39; 95% CI = 1.09–1.79). The unadjusted OR for MCI tended to increase according to the severity of hyperhomocysteinemia (OR = 1.39; 95% CI = 1.09–1.79 vs. OR = 2.61; 95% CI = 1.22–5.61; P = 0.001). Furthermore, these trends did not change after adjustment for serum folate and vitamin B-12. Low concentrations of these vitamins are putative risk factors for cognitive decline.

### Discussion

We found a strong and graded association between plasma tHcy levels and the risk of MCI in an elderly Korean population. Plasma tHcy concentration was significantly higher in subjects with MCI compared with normal subjects and the risk of MCI was >2-fold greater in subjects with intermediate hyperhomocysteinemia (>30 μmol/L) than in those without hyperhomocysteinemia (≥15 μmol/L). This association appeared to be independent of other well-known putative risk factors for cognitive decline such as age, sex, education, smoking, marital status, and serum vitamin levels. Our results suggest that hyperhomocysteinemia may be an independent risk factor for MCI in elderly Korean subjects.

Our finding is consistent with previously reported results (9,14,25). In cross-sectional analyses from the Leiden 85-Plus study in the Netherlands in noninstitutionalized elderly subjects aged 85 years, cognitively impaired individuals had higher serum concentrations of homocysteine than did healthy subjects, as assessed by the Mini-Mental State Examination (MMSE) (9). A similar result was also reported from the longitudinal Veterans Affairs Normative Aging Study, which showed a causal relationship between elevated homocysteine levels and cognitive decline (10).
decline in American men aged 50–85 y (25). Furthermore, a case-control study using an elderly Korean population documented that plasma tHcy concentration was significantly greater in elderly subjects with MCI compared with healthy Korean subjects (14). Moreover, the authors of that study showed that subjects in the highest homocysteine tertile (≥12.3 μmol/L) had a 2.4-fold increase in risk for MCI compared with subjects in the lowest tertile (<10 μmol/L) after adjustment for age, sex, and education level. This result is fairly consistent with our study, showing that a plasma tHcy level in the highest quartile (≥18.45 μmol/L) doubled the risk of MCI, after adjustment for confounding factors, compared with the lowest quartile (≤12.84 μmol/L; data not shown).

Nonetheless, some studies have also shown incompatible results. Among community-dwelling elderly subjects aged ≥55 y from the population-based Rotterdam study in the Netherlands, no relationship was found between tHcy levels and concurrent cognitive impairment, as assessed by the MMSE, or subsequent cognitive decline over 2.7 y, after adjustment for age, sex, and education level (12). Similarly, plasma homocysteine concentration did not differ between normal and MCI elderly subjects, as assessed by the Mayo Clinic criteria in a group of Polish subjects (13).

These inconsistent results may be attributable to differences in the ethnic origin of the study populations. In this regard, ethnic-specific differences in the prevalence of the apoE e4 allele should be considered, as the apoE e4 genotype may be associated with poorer performance in cognitive function, particularly memory (16). For example, the apoE e4 allele has a stronger effect on Alzheimer’s disease in Japanese subjects than in Caucasians (26). None of the studies mentioned above, except for our current study, included apoE genotype as a confounding factor for analysis. Another possible reason for the incompatible results is the use of different criteria for MCI diagnosis, such as the Mayo Clinic criteria (this study) and the MMSE score (9). Petersen et al. (3) reported that the use of alternative criteria for MCI could lead to variable results for MCI outcome because of differences in the interpretation of cognitive impairment. Therefore, a direct comparison is not possible because of the many differences in the study conditions.

Several mechanisms for the effects of homocysteine on cognitive decline have been proposed. Elevated homocysteine levels may induce DNA damage in the central nervous system in addition to being implicated in vascular disease (27,28). Several authors have proposed that homocysteine-related impairment of glutathione metabolism and oxidative stress, impaired DNA methylation, and associated epigenetic mechanisms might increase amyloid-β-peptide production and toxicity as, for example, in Alzheimer’s disease (27–29). Increased concentrations of homocystic acid, an N-methyl-D-aspartate receptor agonist and a metabolite of homocysteine, may result in neuronal dysfunction (30).

We found an inverse association between plasma tHcy and serum folate or vitamin B-12 concentration. This suggests that elevated homocysteine concentration may be reduced through folate or vitamin B-12 supplementation in patients with MCI. Some intervention studies have also suggested a beneficial effect of folate alone or in combination with vitamin B-12 supplementation on cognitive performance (31–33). Despite the correlation between plasma homocysteine and serum vitamin levels, serum folate and vitamin B-12 concentrations in our study did not differ between elderly subjects with and without MCI (Table 1). Overall, our data suggest that a shortage of these 2 vitamins, which are essential factors for the methylation of homocysteine to methionine, may contribute to the increase in plasma homocysteine concentrations rather than directly affect cognitive function. Several studies support this hypothesis (34,35). Oral folate plus vitamin B-12 for 12 wk decreased homocysteine concentration in patients aged ≥65 y with vascular disease but had no beneficial effect on cognitive performance (34). Similarly, vitamin B-12 alone or in combination with folate for 24 wk decreased plasma homocysteine concentration by 36%, but the vitamin supplementation did not improve cognitive function among subjects aged ≥70 y (35). Further research on the association between cognitive performance and folate or vitamin B supplementation should be undertaken in a large population with MCI, using sensitive measures of cognitive function.

Our study had several limitations that should be addressed in future studies. We observed an association between plasma tHcy and risk of MCI only in a cross-sectional setting. Therefore, we were unable to determine whether plasma tHcy is a cause or consequence of MCI. The causal relationship between plasma homocysteine levels and cognitive impairment should be evaluated in a follow-up study in the Korean population. Furthermore, because some data used in the analyses were obtained from self-reports of subjects with MCI, these data may not be as accurate as those of normal subjects. However, the lack of difference in the discrepancy between self- and other-reported everyday functioning in the MCI groups compared with the normal subjects was previously reported (36), suggesting that the effect of any potential recall error from the MCI groups is likely to be small. Interviewer’s help, particularly in the case of self-reported disease history from the MCI groups, may also significantly reduce these recall errors. Nevertheless, our study had several advantages. To date, this study has the largest sample size among studies of elderly subjects and the cohort was based on a stratified and random sample. Furthermore, the majority of putative risk factors for cognitive function, such as serum vitamins and apoE genotype, were included in the analysis.

In conclusion, our results suggest that hyperhomocysteinemia may be an independent risk factor for MCI in elderly Koreans. Large-scale intervention studies are needed to evaluate whether lowering blood homocysteine concentrations reduces the risk of cognitive impairment in elderly subjects.

### Literature Cited


17. Brady SB, van Boxtel MP, Schouten EG, de Groot LC, Bloo RJ, Clarke R, Ueland PM. Homocysteine and mild cognitive impairment 2097


