Through the looking glass: Risk factors, radiological hallmarks and cognitive function in cerebral small vessel disease

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Homocysteine, progression of ventricular enlargement, and cognitive decline. The SMART-MR study

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ABSTRACT

Background Homocysteine may be a modifiable risk factor for cognitive decline and brain atrophy, particularly in older persons. We examined whether homocysteine increased the risk for cognitive decline and brain atrophy, and evaluated the modifying effect of age.

Methods Within the Second Manifestations of ARTerial disease-Magnetic Resonance study – a prospective cohort study among patients with atherosclerotic disease – longitudinal analyses were performed in 663 patients (mean age: 57 ± 9 years; follow-up: 3.9 ± 0.4 years). At baseline and follow-up, brain segmentation on magnetic resonance imaging was used to quantify relative (%) cortical, ventricular, and global brain volumes, and z-scores of memory and executive functioning were calculated. Linear regression analysis was used to estimate associations of homocysteine (per standard deviation increase) and hyperhomocysteinemia (HHCY) with brain volumes, memory, and executive functioning at follow-up, adjusted for baseline brain volume, memory, and executive functioning, respectively, and age, sex, and vascular risk factors. Furthermore, interaction terms between homocysteine and age (continuous) were added.

Results Significant interactions were observed between total plasma homocysteine (tHcy) and age with cortical, ventricular, and global brain volume (for all three measures: p<0.05), and between HHCY and age with executive functioning (p<0.04), and results were stratified by age. In patients aged ≥65 years, increasing tHcy level and HHCY were significantly associated with progression of ventricular enlargement (B=0.07%, 95% confidence interval [CI]: 0.01% to 0.13% and B=0.16%, 95% CI 0.01% to 0.31%, respectively) and with a decline in executive function (B=-0.29, 95% CI -0.54 to -0.04 and B=-0.84, 95% CI -1.37 to -0.32, respectively).

Conclusion Elevated tHcy was related to progression of ventricular enlargement and increased the risk for a decline in executive functioning in older persons.
INTRODUCTION

Due to the aging population, dementia will become one of the major health problems in the near future. Early recognition of those at risk could help to identify patients for preventive treatment. Cognitive impairment and brain atrophy are commonly used as early markers of dementia. Consequently, there is a need to identify potentially modifiable risk factors for cognitive impairment and brain atrophy. Homocysteine – an amino acid formed during methionine metabolism – may be such a risk factor, as it is associated with cognitive impairment, brain atrophy, and dementia. Moreover, homocysteine levels can be decreased by folic acid, vitamin B6, and B12, and therefore could be a potential target for therapy.

Many cross-sectional and several longitudinal studies examined whether increased homocysteine levels were associated with cognitive impairment. Although not all, most showed a positive association. It is thought that brain atrophy may be an intermediate in the association. Cross-sectional studies found that an increased total plasma homocysteine (tHcy) is associated with decreased total brain volume, hippocampal volume, and cortical volume, and increased subcortical volume. Yet, only one prospective study examined the association between tHcy and progression of brain atrophy, and no study examined both progression of brain atrophy and cognitive decline.

We aimed to examine the prospective association of plasma homocysteine with progression of global and cortical brain atrophy and ventricular enlargement, using automated quantitative brain volume measurements, in persons with symptomatic atherosclerotic disease. Further, we examined the prospective association of homocysteine with risk for cognitive decline.

METHODS

Data were used from the Second Manifestations of ARTerial disease-Magnetic Resonance (SMART-MR) study, a prospective cohort study aimed to investigate brain changes on magnetic resonance imaging (MRI) in 1309 independently living patients presenting with symptomatic atherosclerotic disease. In brief, from 2001 to 2005, all patients newly referred to the University Medical Center Utrecht with manifest coronary artery disease, cerebrovascular disease, peripheral arterial disease, or an abdominal aortic aneurysm (AAA) and without magnetic resonance (MR) contraindications were invited to participate. During a 1-day visit to our medical center, an MRI of the brain was performed, in addition
to a physical examination, ultrasonography of the carotid arteries, and blood and urine sampling. Risk factors, medical history, and functioning were assessed with questionnaires that the patients completed before their visit to the medical center. Neuropsychological testing was introduced in the SMART-MR study starting in January 2003 and was performed on the same day as the MRI and other investigations.

From 2006 to 2009, all participants still alive were invited for follow-up, including an MRI of the brain and neuropsychological testing. In total, 754 individuals of the surviving cohort (61% of n51238) gave written informed consent and participated at follow-up. The SMART-MR study was approved by the ethics committee of our institution, and written informed consent was obtained from all participants.

**MRI protocol**

At baseline and follow-up, the MR investigations were performed on a 1.5-T whole-body system (Gyroscan ACS-NT, Philips Medical Systems, Best, The Netherlands). The protocol consisted of transversal T1-weighted (repetition time [TR]/echo time [TE]: 235/2 ms; flip angle, 80°), T2-weighted (TR/TE: 2200/11 ms and 2200/100 ms; turbo factor, 12), fluid-attenuated inversion recovery (FLAIR) (TR/TE/inversion time: 6000/100/2000 ms), and inversion recovery (TR/TE/inversion time: 2900/22/410 ms) sequences (field of view, 230 x 230 mm; matrix size, 180 x 256; slice thickness, 4.0 mm; no gap; 38 slices).

**Brain segmentation**

We used the T1-weighted gradient-echo, inversion recovery sequence and FLAIR sequence for brain segmentation. The probabilistic segmentation technique has been described elsewhere,\(^\text{18,19}\) and has been proven to be very reliable, with similarity indices exceeding 0.8 for all segmented tissue and cerebrospinal fluid (CSF) volumes, indicating an excellent agreement between the results of the segmentation program and manual segmentation. Two preprocessing steps were performed. The first step was an intrapatient rigid registration to compensate for motion and scan variations.\(^\text{20}\) The second preprocessing step was an automatic skull-stripping of the T1 image\(^\text{21}\) to define a proper region of interest for the segmentation process.

The actual segmentation of the MR images was performed by k-nearest neighbor classification.\(^\text{18}\) The result of the classification method is a probability value for each voxel that quantifies the amount of a specific tissue type contained in that voxel. Total volumes
were calculated by adding all probabilities and multiplying this sum with the volume of 1 voxel. The segmentation program distinguishes gray matter, white matter, sulcal and ventricular CSF, and lesions. The results of the segmentation analysis were visually checked for the presence of infarcts and adapted, if necessary, to make a distinction between white matter lesions (WMLs) and infarct volumes. Total brain volume was calculated by summing gray matter, white matter, WML, and infarct volumes. Total intracranial volume (ICV) was calculated by summing the total brain volume and the volumes of the sulcal and ventricular CSF. The brain volumes used for this analysis were brain parenchymal fraction (BPF), cortical gray matter fraction (GMF), and ventricular fraction (VF), as indicators of global, cortical, and subcortical atrophy (ventricular enlargement). All brain volumes were normalized for ICV.

**Brain infarcts and WMLs**

At baseline and follow-up, the whole brain was visually searched for infarcts by an investigator and a neuroradiologist. Discrepancies in rating were re-evaluated in a consensus meeting. Raters were blinded to all clinical information. Infarcts were defined as focal hyperintensities of at least 3 mm diameter on T2-weighted images. Hyperintensities located in the white matter also had to be hypointense on T1-weighted and FLAIR images to distinguish them from WML. Dilated perivascular spaces were distinguished from infarcts on the basis of their location, form, and absence of gliosis. The location, affected flow territory, and type were scored for every infarct. Brain infarcts were categorized as cortical infarcts, lacunar infarcts, large subcortical infarcts, and infratentorial infarcts. We defined lacunar infarcts as infarcts of 3 to 15 mm diameter and located in the subcortical white matter, thalamus, or basal ganglia. Large subcortical infarcts were sized >15 mm and were not confluent with cortical infarcts. Infratentorial infarcts were located in the brain stem or cerebellum.

Volumes of WML obtained with the segmentation program consisted of deep and periventricular WML volumes and were summed to obtain the total volume of WML. WML volumes were normalized for ICV and natural log-transformed.

**Neuropsychological assessment**

Memory and executive functioning were assessed with neuropsychological tests, sensitive to mild impairments. We calculated z-scores (individual test score minus mean test score divided by the standard deviation [SD]) for the neuropsychological tests at baseline and at follow-up using the mean and SD of the baseline tests. Composite z-scores for memory and executive functioning were computed by averaging the z-scores of all subtests per domain.
Verbal memory was assessed with five consecutive trials of the 15-word learning test (a modification of the Rey Auditory Verbal Learning test). Immediate recall and delayed recall were assessed. Nonverbal memory was assessed using the delayed recall condition of the Rey–Osterrieth Complex Figure test.

Executive functioning was assessed with three tests. The Visual Elevator test – a subtest of the Test of Everyday Attention – is a timed test of 10 trials that measures mental flexibility and shifting of attention. The Brixton Spatial Anticipation test was used to assess the capacity to discover logical rules and mental inhibition and flexibility. The total number of errors made was scored. The Verbal Fluency test (letter A, 1 minute time frame) was used to assess mental flexibility and employment of strategies. Before calculating the z-scores, the scores of the Visual Elevator test and Brixton Spatial Anticipation test were multiplied by 21 so that lower scores represented poorer performance.

Premorbid intellectual functioning was assessed by using the Dutch version of the National Adult Reading Test (DART). Educational level was divided into seven categories, graded from primary school to academic degree, according to the Dutch educational system.

**Vascular risk factors and homocysteine**

An overnight fasting venous blood sample was taken to determine glucose, creatinine, and lipid levels. Height and weight were measured without shoes and heavy clothing, and the body mass index was calculated (kg/m²). Systolic and diastolic blood pressures (mm Hg) were measured twice with a sphygmomanometer and averaged. Diabetes mellitus was defined as a known history of diabetes, a fasting glucose level of 7.0 mM/L, or self-reported use of glucose-lowering agents. Estimated glomerular filtration rate was computed with the Cockroft–Gault equation. Smoking habits and alcohol intake were assessed with questionnaires. The number of pack-years of smoking was calculated, and alcohol intake was categorized as never, former, or current. Ultrasonography was performed to measure the intima-media thickness (mm) in the left and right common carotid arteries, represented by the mean value of six measurements. Homocysteine was determined in a blood sample that was drawn at inclusion after overnight fasting of at least 8 hours. The method of Shipchandler and Moore was used to analyse tHcy levels. Hyperhomocysteinemia (HHCY) was defined – according to sex-specific 95th percentiles studied by Van der Griend et al. – as a fasting tHcy level of 16.3 μM/L or greater in women and 18.8 μM/L or greater in men.
Study sample

Of the 1309 patients, 19 had no MRI and 14 had no FLAIR sequence. In addition, in 44 patients, brain volume data were missing due to motion or artifacts. Of the 1232 patients, 718 participated in the follow-up examination. Because 38 patients had no MRI and brain volume data were missing due to motion or artifacts in 17 patients, the analytical sample consisted of 663 patients. For the analyses on cognition as the outcome, we had data in a subset of 416 patients (due to the fact that cognitive testing was not introduced until 2003).

Statistical analysis

Missing data rarely occur completely at random, and a complete case analysis (omission of all participants with one or more missing values) leads to loss of statistical power and to biased results. Furthermore, the advantage of multiple imputation over single or mean imputation is that it does not reduce the variation in the data. In fact, variation is added to the data, of which the net effect is that the amount of variation is similar to the situation when the full data would have been observed. We, therefore, used multiple imputation (10 data sets) to address baseline missing values in homocysteine level, demographics, vascular risk factors, WML, and brain atrophy using the open source statistical programme R (aregImpute) (version 2.10.0). Percentages of missing values before imputation are given in Table 3.1 footnote. Data were analyzed using SPSS version 17.0 (Chicago, IL) by pooling the 10 imputed data sets.

Baseline characteristics were calculated for the SMART-MR sample, with follow-up measurements for patients with and without HHCY. Linear regression analysis and analysis of covariance were used to investigate the prospective association between homocysteine and change in measures of brain atrophy (global and cortical atrophy and ventricular enlargement), and change in z-scores for cognitive functioning, by using brain volume measures and the z-scores for cognition at follow-up as the dependent variables and brain volume measures and the z-scores for cognition at baseline as independent variables. tHcy was entered as a continuous variable in the regression analysis, and HHCY as a dichotomous variable in the regression analysis and analysis of covariance. The analyses of homocysteine with change in brain atrophy were corrected for age, sex, and follow-up time (model 1), and the analysis of homocysteine with change in cognition were corrected for age, sex, follow-up time, education, and DART score. To examine whether the association between homocysteine and change in brain atrophy/cognition was explained by vascular risk factors, we further adjusted for body mass index, alcohol consumption, smoking, systolic blood
pressure, diastolic blood pressure, diabetes mellitus, cholesterol level, creatinine clearance (Cockcroft), carotid intima-media thickness, and history of cerebrovascular disease, coronary artery disease, or peripheral artery disease (model 2). As homocysteine may influence brain atrophy and cognition, especially in the elderly population, statistical interaction was assessed by including age (continuous), homocysteine (tHcy or HHCY), and their product term in the linear regression model (model 2), and the analyses were stratified by age (<65 and ≥65 years).

As presence of large brain infarcts may confound the association between homocysteine and change in brain atrophy/cognition, additional adjustments were made for progression of cortical and large subcortical brain infarcts (yes or no). In addition, to investigate whether cerebral small-vessel disease (CSVD) mediates or confounds the association of homocysteine with change in brain atrophy/cognition, we repeated the analyses with additional adjustments for progression of WML volume (change in WML volume) and progression of lacunar infarcts (yes or no) between baseline and follow-up. Finally, to investigate whether brain atrophy mediated the association between homocysteine and cognitive functioning, progression of brain atrophy measures were added to the model.

**RESULTS**

The mean (SD) age of the study population was 58 (10) years. The mean (SD) tHcy was 13 (4) µM/L for men and 12 (4) µM/L for women. Ten percent of the patients had HHCY. Patients with HHCY were older, were more often male, consumed less alcohol, and had worse creatinine clearance (Table 3.1).

**Homocysteine and progression of brain atrophy**

Overall, there were no significant associations between tHcy and progression of global brain atrophy (model 2; BPF: B=0.05%, 95% CI -0.03 to 0.13), ventricular enlargement (VF: B=0.00%, 95% CI -0.02 to 0.02), or cortical atrophy (GMF: B=0.03%, 95% CI -0.14 to 0.20). The p-values of the interaction terms age-by-tHcy for progression of BPF, VF, and GMF in model 2 were p=0.007, p=0.027, and p=0.014, respectively, and for age-by-HHCY were p=0.008, p=0.242, and p=0.023, respectively. When we stratified participants in two age-groups (<65 and ≥65 years) (Table 3.2), we found that in patients aged ≥65 years, increasing levels of homocysteine (per SD) as well as HHCY were associated with a small, but statistically significant, increase in ventricular enlargement (Table 3.2, model 2).
Additional adjustments for progression of large brain infarcts did not change the results (data not shown). Furthermore, adjustments for progression of WML volume and lacunar infarcts slightly attenuated the results (tHcy: β=0.06, 95% CI 0.00 to 0.13, and HHCY: β=0.14, 95% CI -0.01 to 0.29), suggesting that the association of tHcy or HHCY with progression of ventricular enlargement was only partly explained by progression of CSVD. No significant associations were found between homocysteine and progression of global or cortical brain atrophy within the two age strata (Table 3.2).

Table 3.1  Baseline characteristics of the study sample (n=663) according to presence or absence of hyperhomocysteinemia (HHCY)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non HHCY (n=594)</th>
<th>HHCY (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs) †</td>
<td>57 (9)</td>
<td>62 (10)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>107 (18%)</td>
<td>17 (25%)</td>
</tr>
<tr>
<td>Education †</td>
<td>3 (1–6)</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>Dutch version of the National Adult Reading Test †</td>
<td>81 (59–96)</td>
<td>82 (56–98)</td>
</tr>
<tr>
<td>Cerebrovascular disease, n (%)</td>
<td>132 (22%)</td>
<td>21 (30%)</td>
</tr>
<tr>
<td>Coronary heart disease, n (%)</td>
<td>373 (63%)</td>
<td>37 (54%)</td>
</tr>
<tr>
<td>Peripheral artery disease, n (%)</td>
<td>108 (18%)</td>
<td>12 (17%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>94 (16%)</td>
<td>12 (17%)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L) †</td>
<td>4.8 (1.0)</td>
<td>4.9 (1.0)</td>
</tr>
<tr>
<td>Homocysteine (µmol/l) †</td>
<td>12 (3)</td>
<td>22 (4)</td>
</tr>
<tr>
<td>eGFR †</td>
<td>96 (25)</td>
<td>79 (29)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg) †</td>
<td>140 (20)</td>
<td>141 (17)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) †</td>
<td>82 (10)</td>
<td>81 (10)</td>
</tr>
<tr>
<td>Body mass index (kg/m²) †</td>
<td>27 (4)</td>
<td>27 (3)</td>
</tr>
<tr>
<td>Smoking (pack-years) †</td>
<td>20 (0–49)</td>
<td>19 (0–53)</td>
</tr>
<tr>
<td>Current alcohol use, n (%)</td>
<td>478 (81%)</td>
<td>42 (61%)</td>
</tr>
<tr>
<td>Intima Media Thickness (mm) †</td>
<td>0.92 (0.30)</td>
<td>0.99 (0.29)</td>
</tr>
<tr>
<td>White matter lesion volume (% ICV) †</td>
<td>0.09 (0.03–0.35)</td>
<td>0.14 (0.03–0.81)</td>
</tr>
<tr>
<td>Lacunar infarcts (≥1), n (%)</td>
<td>93 (16%)</td>
<td>21 (30%)</td>
</tr>
</tbody>
</table>

Note. Percentage of missing values before imputation—education: 2.3%; DART score: 1.3%; diabetes mellitus: 1.9%; cholesterol: 1.4%; homocysteine: 0.8%; eGFR: 1.3%; smoking and alcohol use: 0.6%; BMI, SBP, and DBP: 0.1%; IMT: 2.1%; cortical gray matter volume: 15.3%; WML volume: 5.9%; all other variables: 0.0%; † mean (SD), ‡ median (10th, 90th percentile), eGFR: estimated Glomerular Filtration Rate (Cockcroft-Gault formula), a Educational level was divided in seven categories, graded from primary school to academic degree, according to the Dutch educational system.

Dutch version of the National Adult Reading Test (range 0–100).
Table 3.2 Linear regression analysis with homocysteine level and change in brain atrophy, stratified by age

<table>
<thead>
<tr>
<th></th>
<th>Global brain atrophy – BPF (%)</th>
<th>Subcortical brain atrophy – VF (%)</th>
<th>Cortical brain atrophy – GMF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;65 years (n=501) B (95% CI)</td>
<td>≥65 years (n=162) B (95% CI)</td>
<td>&lt;65 years (n=501) B (95% CI)</td>
</tr>
<tr>
<td>Homocysteine level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(per SD increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.07 (-0.02; 0.16)</td>
<td>-0.10 (-0.27; 0.07)</td>
<td>0.04 (-0.02; 0.09)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.09 (-0.00; 0.18)</td>
<td>-0.03 (-0.24; 0.17)</td>
<td>0.07 (0.01; 0.13)*</td>
</tr>
<tr>
<td>HHCY (yes vs no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.23 (-0.08; 0.54)</td>
<td>-0.31 (-0.74; 0.12)</td>
<td>0.08 (-0.06; 0.22)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.28 (-0.03; 0.59)</td>
<td>-0.24 (-0.73; 0.25)</td>
<td>0.16 (0.01; 0.31)*</td>
</tr>
</tbody>
</table>

One SD = 4.3 µmol/L. Higher VF means more subcortical brain atrophy, lower GMF and BPF means more cortical and global brain atrophy.

Model 1: age, sex, baseline brain volume (BPF, VF or GMF), and follow-up time.
Model 2: Model 1 + additional adjustments for BMI, systolic blood pressure, diastolic blood pressure, cholesterol level, smoking, alcohol usage, eGFR, carotid IMT, diabetes mellitus, cerebrovascular disease, coronary disease and peripheral artery disease.

* p<0.05.
Homocysteine and cognitive decline

Overall, there were no significant associations between tHcy and a change in z-score for executive functioning (model 2; B=-0.01, 95% CI -0.08 to 0.05) or memory function (B=-0.05, 95% CI -0.11 to 0.02). The p-values of the interaction terms age-by-tHcy for change in executive function and memory function in model 2 were p=0.213 and p=0.923, respectively, and for age-by-HHCY, these were p=0.038 and p=0.380, respectively. When we stratified the participants in two age-groups we found that in patients aged ≥65 years, increasing levels of homocysteine (per SD) as well as HHCY were associated with a significant decline in z-score for executive functioning (Table 3.3, model 2 and Figure 3.1). Furthermore, adjustments for progression of large brain infarcts did not materially change the results (data not shown). Adjustments for progression of CSVD (change in WML volume and lacunar infarcts) somewhat attenuated the associations (tHcy: B=-0.21, 95% CI -0.46 to 0.04, and HHCY: B=-0.65, 95% CI -1.18 to -0.12). When including both progression of ventricular enlargement and progression of CSVD in the model, the associations further attenuated (model 2; tHcy: B=-0.19, 95% CI -0.44 to 0.05, and HHCY: B=-0.59, 95% CI -1.13 to -0.04), suggesting that the association of tHcy or HHCY with decline in executive functioning was partly explained by progression of CSVD and ventricular enlargement.

No significant associations were found between HHCY and a change in z-score for memory function.

DISCUSSION

In this population with symptomatic atherosclerotic disease, increasing homocysteine levels, and in particular HHCY, were associated with progression of ventricular enlargement and with a decline in executive functioning in persons aged ≥65 years.

To our knowledge, this is the first study that examined the prospective association between homocysteine and quantitative measures of cortical brain atrophy and ventricular enlargement as well as cognitive decline in one study population. Most previous cross-sectional population-based studies reported that increasing homocysteine levels were associated with more brain atrophy. However, limited conclusions can be drawn from these studies because of their cross-sectional design, lack of distinguishing between cortical and subcortical brain atrophy, small sample sizes, or use of semiquantitative MRI techniques. Only one previous prospective study was performed using voxel-based morphometry, which found an association between higher levels of homocysteine...
Table 3.3  Linear regression analysis with baseline total homocysteine level and change in z-score for executive functioning, in the total population (n=416) and stratified by age

<table>
<thead>
<tr>
<th></th>
<th>Z-score for executive function</th>
<th>&lt;65 yrs (n=315)</th>
<th>≥65 yrs (n=101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine level (per SD increase)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.03 (-0.10 to 0.04)</td>
<td>-0.18 (-0.38 to 0.03)</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.00 (-0.07 to 0.07)</td>
<td>-0.29 (-0.54; -0.04)*</td>
<td></td>
</tr>
<tr>
<td>HHCY (yes vs no)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.17 (-0.39 to 0.05)</td>
<td>-0.70 (-1.17 to -0.22)</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.12 (-0.34 to 0.10)</td>
<td>-0.84 (-1.37 to -0.32)*</td>
<td></td>
</tr>
</tbody>
</table>

One SD = 4.3 µmol/L.
Model 1: Adjusted for age, sex, education, the Dutch version of the National Adult Reading Test, baseline cognitive z-scores, and follow-up time.
Model 2: Model 1 + adjustments for BMI, systolic blood pressure, diastolic blood pressure, cholesterol level, smoking, alcohol usage, eGFR, carotid IMT, diabetes mellitus, cerebrovascular disease, coronary disease and peripheral artery disease.
* p<0.05.

Figure 3.1  Longitudinal relation between homocysteine and change in cognitive functioning, stratified by age.
Bars represent the adjusted mean (95% CI) change in cognitive functioning after 3.9 years of follow-up (model 2). NHHCY = no hyperhomocysteinemia, HHCY = hyperhomocysteinemia. * p<0.01.
and progression of white matter atrophy.\textsuperscript{15} An explanation for the association between increasing tHcy levels and progression of ventricular enlargement might be that white matter may be more vulnerable than gray matter to the excitatory and vascular damage produced by homocysteine.\textsuperscript{15,39} Yet, the exact mechanisms need to be established. A few studies examined whether WML was an intermediate in the association between homocysteine and progression of brain atrophy; however, in accordance with our findings, they reported this could not fully explain the association, suggesting this only partly mediates the association.\textsuperscript{7,15} Possibly, MRI-invisible microangiopathy may also intermediate the association between homocysteine and ventricular enlargement.\textsuperscript{40}

Although not all, most previous cross-sectional and longitudinal studies showed that homocysteine was a risk factor for cognitive decline in several domains, including executive functioning, constructional praxis, and memory.\textsuperscript{4,10-12} We found that in our nondemented atherosclerotic patients aged ≥65 years, especially executive functioning was affected. When we examined whether progression of CSVD mediated the association, we found this could partly explain the association. We additionally examined whether progression of ventricular enlargement was an intermediate, and found that the association further attenuated. This suggests that both vascular and degenerative mechanisms may partly explain the association between homocysteine and cognitive functioning in the elderly population, but other intermediates need to be determined.

Age appeared to be an effect modifier in our study. In patients aged ≥65 years, an association was found, in particular, with HHCY and progression of ventricular enlargement and executive functioning. It is likely that any effect of homocysteine leading to cognitive decline or brain atrophy is a slow process and becomes evident later. It might, therefore, especially be seen in older patients, which is shown in several, but not all, other studies.\textsuperscript{12,15,34,41} In patients aged <65 years, a nearly significant association was found between tHcy and less progression of global brain atrophy. Although we do not have an explanation readily available, a possible reason could be selective nonparticipation, with younger, healthier patients with larger brain volumes participating in the study.

Strengths of the study include the large number of patients investigated and the volumetric assessment of measures of brain atrophy, which made it possible to obtain precise estimates of progression of brain atrophy, and resulted in a large power to detect associations. Furthermore, the segmentation of different brain tissue types and CSF spaces allowed us to differentiate between cortical brain atrophy and ventricular enlargement. Finally, the extensive information available on (vascular) risk factors and vascular disease made it possible to adjust for potential shared risk factors and extent of atherosclerosis.
Our study had limitations. First, we used a single determination of homocysteine concentration. The within-person variability in tHcy tends to underestimate the real association between the usual level of tHcy and disease rates during follow-up by 10% to 15%. Second, we did not have data on vitamin levels such as folate or vitamin B12; however, studies that corrected for vitamin use found that the association between tHcy and brain atrophy or cognition did not materially change. Furthermore, some patients may have lowered their tHcy levels by taking vitamin B12 and folic acid, as this advice was given during the first years of the SMART-MR study to patients who had HHcy at baseline. This may have underestimated the observed association. Third, the association between tHcy and progression of hippocampal volume was not determined in the SMART-MR study, so we could not include this in the analyses. However, because we found that especially a change in executive functioning and ventricular enlargement were influenced by an elevated homocysteine, we do not expect a strong association of tHcy with hippocampal atrophy, as this is mainly associated with memory function. Fourth, persons who participated in this study were healthier than those who did not undergo a follow-up examination. This may have led to an underestimation of the observed associations in patients aged ≥65 years. Finally, as the majority of our study sample consisted of patients with symptomatic atherosclerotic disease, our results may not be generalizable to the general population.

Because elevated homocysteine levels are treatable, early preventive interventions could be important. Two recent randomized controlled trials found that homocysteine-lowering treatment showed modest positive effects on cognition and slowed the rate of brain atrophy in elderly patients presenting with mild cognitive impairment.

In conclusion, in this population with atherosclerotic disease, older persons with an elevated homocysteine level had greater progression of ventricular enlargement and decline in executive functioning, which were partly mediated by progression of CSVD. The impact of high tHcy levels on the brain becomes evident after middle age, and remediation should, therefore, begin at an early age, much before symptoms become apparent.

REFERENCES


