Cognitive decline in late-life: biological markers and early identification of persons at risk for dementia

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Tessa Nicole van den Kommer

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Cognitive decline in late-life: biological markers and early identification of persons at risk for dementia

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ter verkrijging van de graad Doctor aan
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door

Tessa Nicole van den Kommer

geboren te Delft

promotoren:	prof.dr. D.J.H. Deeg
	prof.dr. C. Jonker
copromotoren:	dr. M.G. Dik
	dr. H.C. Comijs

“ ... It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of light, it was the season of darkness, it was the spring of hope, it was the winter of despair ...”

Charles Dickens, A Tale of Two Cities
English novelist (1812 - 1870)

Contents


Chapter 7

Homocysteine and inflammation: predictors of cognitive decline in older persons?

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Abstract

The aim of the current study was to examine the association between homocysteine and six-year cognitive decline, and the modifying role of the inflammatory markers Interleukin-6 (IL-6), C-reactive protein (CRP) and α1-antichymotrypsin (ACT). Data were collected within the Longitudinal Aging Study Amsterdam (ages ≥ 65 years) and analyzed using multiple longitudinal regression models (N = 1,257 of whom N = 1,076 had longitudinal data). Cognition was measured with the Mini-Mental State Examination (general cognition), Auditory Verbal Learning Test (memory), Coding Task (information processing speed) and Raven's Coloured Progressive Matrices (fluid intelligence).

Higher homocysteine at baseline was negatively associated with prolonged lower cognitive functioning and a faster rate of decline in information processing speed and fluid intelligence. The negative association between higher homocysteine and immediate recall was strongest in persons with a high level of IL-6. Only in the highest tertile of CRP, higher homocysteine was negatively associated with retention. In the middle tertile of ACT, higher homocysteine was associated with lower information processing speed and faster decline. Both in the lower and middle tertile of CRP, higher homocysteine was associated with a faster rate of decline in information processing speed. The results implicate that a combination of both risk factors may be used as a marker for cognitive impairment.

Keywords: Homocysteine; Cognitive decline; Inflammation; Longitudinal population-based study.

Introduction

Elevated levels of homocysteine, and enzymatic defects or vitamin deficiencies causing inhibition of homocysteine metabolism (Selhub and Miller, 1992) have been linked to cognitive impairment and dementia. Evidence for this association has been provided mainly by cross-sectional studies (Elias et al., 2005; Feng et al., 2006; Prins et al., 2002; Quadri et al., 2004; Ravaglia et al., 2003), although not all studies have shown an association between elevated total serum homocysteine (tHcy) and cognitive impairment or dementia (Miller et al., 2002; Ravaglia et al., 2000). Longitudinal studies have shown even more conflicting results. While the suggestion that elevated tHcy is an independent risk factor for developing cognitive decline or dementia over time has been confirmed by several longitudinal studies that have been undertaken so far (Clarke et al., 1998; Dufouil et al., 2003; Haan et al., 2007; McCaddon et al., 2001; Ravaglia et al., 2007a; Seshadri et al., 2002; Teunissen et al., 2003; Tucker et al., 2005), other prospective studies failed to detect an association (Kado et al., 2005; Kalmijn et al., 1999; Luchsinger et al., 2004; Mooijaart et al., 2005). These inconsistent findings might be due to differences in study population (type and severity of dementia and cognitive deficits), differences in sensitivity of applied cognitive measures, measurement of biomarkers in non-fasting state, and the inclusion of confounding factors in the statistical analyses.

Although several mediating or modifying mechanisms that may underlie the negative effect of elevated tHcy on cognitive functioning and the central nervous system have been implied, still no definitive answers exist. One potentially important mechanism that is increasingly linked to dementia and cognitive decline is (systemic) inflammation (Dik et al., 2005; Engelhart et al., 2004; Yaffe et al., 2003). In turn, hyperhomocysteinemia has been associated with increased inflammation (Bates et al., 1997; Gori et al., 2005; Ravaglia et al., 2004), although some studies have shown no association (Kelly et al., 2004; Nilsson et al., 2002). Both elevated levels of tHcy and inflammation have adverse effects on the vascular system and are risk factors for atherosclerosis. Furthermore, atherosclerosis is increasingly known to be linked to dementia (Newman et al., 2005; Van Oijen et al., 2007). A synergistic effect of both factors could be hypothesized, leading to (faster) cognitive decline and dementia (Gunstad et al., 2006; Papatheodorou and Weiss, 2007). So far, the association between both inflammation and tHcy, and cognitive decline has not been studied intensively. To our knowledge only one prospective study has focused on the association between dementia and both inflammatory markers and hyperhomocysteinemia (Ravaglia et al., 2007b). However, the authors did not examine the possible interaction between both factors, but focused solely on the independent relationships and possible confounding effects. They found that high serum C-reactive protein (CRP) levels, especially in association with concurrent high serum Interleukin-6 (IL-6) levels was a significant predictor of the risk of vascular dementia (VaD) independently of hyperhomocysteinemia. In contrast, hyperhomocysteinemia was solely associated with increased risk of Alzheimer's disease (AD), whereas none of the inflammatory markers was a significant predictor of AD.

In the present longitudinal population-based study we examined the association between tHcy and cognitive decline in several cognitive domains over six years of follow-up, and the possible interaction between tHcy and the inflammatory markers IL-6, CRP and α1-antichymotrypsin (ACT).

Methods

Study sample

Subjects were participants in the Longitudinal Aging Study Amsterdam (LASA), an ongoing population-based study in the Netherlands. Procedures regarding sampling and data collection have previously been described in detail (Deeg et al., 2002). In summary, a random sample of older men and women, stratified by age and sex according to the expected five-year mortality, was drawn from the population registries of eleven municipalities in three geographic areas of the Netherlands. Data collection started in 1992/1993, and included follow-up measurements every three years. Interviews were conducted in the homes of the respondents and consisted of a main and a medical interview in which tests were performed and structured questionnaires were completed. Main interviews were done by specially trained and intensively supervised interviewers, while nurses conducted the medical interviews. Informed consent was obtained from all respondents, and the study was approved by the Ethical Review Board of the VU University Medical Center (VUmc).

In total, 3,107 subjects were enrolled in the LASA study. During the second measurement in 1995/1996, 2,545 of the 3,107 respondents (81.9%) participated in the main interview. Loss to follow-up was mainly due to mortality. Of the 562 subjects who were lost to follow-up, 416 died (13.4%), 90 refused (2.9%), 38 were ineligible (1.2%), and 18 could not be contacted (0.6%). For the present study subjects were included aged 65 years and older (N = 1,509), of whom blood samples were obtained during the second data collection (N = 1,331) and tHcy levels could be determined (N = 1,301). Blood samples were obtained in the VUmc (respondents living in Amsterdam and vicinity), or a health care center near the respondents' home (respondents living in Zwolle or Oss and vicinity). For respondents unable to come to the VUmc or a health care center near their home, blood samples were drawn in the home of the participant. Participants with vitamin B_{12} levels above 800 pmol/L (N = 32) were excluded from analyses since these high values reflect vitamin supplementation and thus might represent a bias with respect to the association under study. In addition, persons with creatinine levels above 200 µmol/L (N = 12), indicating renal dysfunction, were excluded from the study (Dhonukshe-Rutten et al., 2005) to rule out the possibility that these outliers would bias the model. This resulted in a study sample of 1,257 subjects. The respondents participating in the medical interview who refused to take part in the blood drawing procedure, were significantly older and had lower scores on cognition (all $p < 0.0001$) compared with subjects of whom blood samples were available. Of the 1,257 respondents with valid tHcy data, 1,076 also participated in the three-year follow-up interview (85.6%). Figure 1 shows a flow chart of the study sample. Of the 181 subjects

who were lost to follow-up, 153 died (12.2%), 11 refused (0.9%), 11 were ineligible (0.9%), and 5 could not be contacted (0.5%). Of the 1,076 respondents participating in the three-year follow-up, 895 participated in the interviews during the six-year follow-up (83.2%). Of those lost to follow-up, 161 died (15.0%), 9 refused (0.8%), 10 were ineligible (0.9%) and 1 could not be contacted (0.1%). Subjects who were lost to follow-up were significantly older, had lower scores on cognition, and were more likely to be men (all $p < 0.001$). Furthermore, respondents lost to follow-up had significantly higher tHcy levels at baseline ($p < 0.001$).

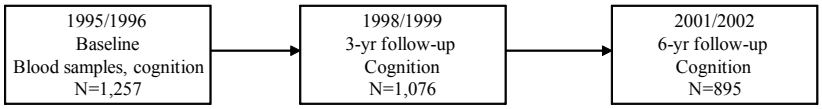

Figure 1 Flow chart of the study sample

Cognitive functioning

Objective cognitive tasks were selected which measure cognitive functions sensitive to decline with aging, and which can be used for screening of cognitive dysfunction and dementia.

General cognitive functioning was measured with the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). The MMSE is a widely applied, brief instrument used for screening of cognitive impairment. Scores range from 0 to 30, a higher score indicating better performance. Memory was measured with an abbreviated version of the Auditory Verbal Learning Test (AVLT) (Rey, 1964). The abbreviated AVLT consists of three learning trials instead of five to reduce the burden for the respondent. In each trial, a list of 15 words is read out loud by the interviewer, after which the respondent is asked to sum up as many words as they can remember. Immediate recall is defined as the highest score out of three trials (range, 0-15). After a distraction period of approximately 20 minutes, the respondent is asked to recall as many words as possible (delayed recall, range 0-15). The retention score is defined as the percentage of the score on immediate recall retained after 20 minutes ((delayed recall/immediate recall) x 100%). Immediate recall and retention were analyzed. At follow-up, a parallel version of the AVLT was used. The parallel versions, which are used in treatment-research (Moller et al., 1998), were validated and tested on parallelism (Jolles et al., 1995). Information processing speed was measured by an adapted version of a letter substitution task, the Alphabet Coding Task-15 (Piccinin and Rabbitt, 1999). During this timed task the respondent is asked to combine as many characters as possible according to a given example (the substitution key). The substitution key shows 15 combinations of 2 characters in a row of double boxes. The test itself shows rows of double boxes, in which only the upper box contains characters and the lower box is empty. The respondent has to name the missing characters corresponding to the characters in the upper box (using the substitution key) as quickly and accurately as possible. The task consists of three

identical one-minute trials. The score on each trial consists of the number of correctly completed characters. The mean score of the three trials was used in the analyses. Fluid intelligence, defined as the ability to deal with new information, was assessed with the Raven's Coloured Progressive Matrices (RCPM) (Raven, 1984). The RCPM is a non-verbal visual test, which assesses the ability for non-verbal, abstract reasoning, consisting of three sections (A, Ab and B) each containing 12 items. The respondent is presented with an incomplete design (matrix) and six alternatives from which one has to be chosen to complete the design. The items increase in difficulty, and so do the three sections. In LASA subset A and B were used to reduce test burden for the respondent. Previously it has been shown that the total score on A, Ab and B correlates strongly (r = 0.96) with the total score on sections A and B (Van den Heuvel and Smits, 1994). Scores (section A and B) range from 0 to 24, a higher score indicating a better performance.

Measurement of tHcy levels

Morning blood samples were obtained, and were kept deep-frozen until analysis. Subjects were allowed to eat toast or drink tea, but no dairy products. EDTA plasma samples were analyzed for tHcy with the Abbott IMx analyzer at the Laboratory of Clinical Chemistry of the VUmc. The IMx method uses fluorescence polarization immunoassay (FPIA) technology.

Potential modifiers

The serum levels of ACT, CRP, and IL-6 were determined using sensitive regular immunoassays (ELISA) developed and performed at Sanquin Research, Amsterdam. The IL-6 ELISA was obtained from the Business Unit Immune Reagents of Sanquin, and performed according to manufacturer's instructions. CRP levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as catching antibodies and a biotinylated mAb against CRP (CLB anti–CRP-2) as the detecting antibody (Bruins et al., 1997). ACT was measured with an ELISA in which specific mAbs against ACT were used (Rozemuller et al., 1991). Recombinant IL-6, purified CRP and pooled human plasma were used as standards in the respective assays. Results were expressed as μg/mL for CRP, pg/mL for IL-6, and % of normal plasma for ACT. The normal human plasma pool (% NHP) used as a standard for ACT contained ~300 mg ACT per L. The inter-assay coefficient of variation (CV) was less than 5.2% for ACT, less than 4.2% for CRP, and less than 5% for IL-6. The intra-assay CV was 4.1% for ACT, 3.2% for CRP, and 3.3% for IL-6. The detection limits were 0.8 ng/mL for CRP, and 5 pg/mL for IL-6. All values were measured in duplicate, with averages being reported.

Potential confounders

All analyses were adjusted for time and age. In addition, the following potential confounders were included in the analyses: education, sex, vitamin B_{12}, creatinine, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, fructosamine, Apolipoprotein e4, cardiovascular disease, hypertension, diabetes mellitus,

arthritis, atherosclerosis, use of lipid lowering drugs, use of anti-inflammatory drugs, depressive symptoms, body mass index (BMI), alcohol consumption, smoking, physical activity, IL-6, ACT, and CRP.

The time variable was defined as the number of years (i.e. 0, 3 and 6 years) between the determination of tHcy levels and time of follow-up cycle. Data on age and sex were derived from the population registries at baseline. Education was assessed by asking the respondent for the highest educational level completed, which was converted into the total number of years of education (range, 5-18 years). Serum levels of vitamin B_{12} were determined at the Endocrine Laboratory of the VUmc with a competitive immunoassay luminescence on the automated ACS 180 System (Bayer Diagnostics, Mijdrecht, The Netherlands). Serum creatinine was measured using either the kinetic Jaffe method or the Jaffe alkaline picrate reaction (these are similar methods). Serum total cholesterol, HDL cholesterol and triglycerides were measured with a Hitachi 747 analyzer (VUmc) using enzymatic colorimetry assay (Roche diagnostics, Mannheim, Germany). Fructosamine was determined by a colorimetric test. The inter-assay coefficient of variation (CV) was < 2.8% for fructosamine and triglycerides, and < 6.4% for HDL cholesterol. LDL cholesterol was calculated as: total cholesterol - HDL cholesterol - (0.456 x total triglyceride concentration) (Friedewald et al., 1972). This was done only for triglyceride levels of < 5.0 mmol/L. ApoE phenotype was determined by isoelectric focusing of delipidated serum samples, followed by immunoblotting (Havekes et al., 1987). The distribution of ApoE phenotypes was in Hardy-Weinberg equilibrium (ApoE e2/2: 0.8%; e2/3: 11.7%; e3/3: 60.8%; e2/4: 2.6%; e3/4: 20.4%; e4/4: 3.2%; missing: 0.6%). ApoE status was classified as e4 carriers for subjects with the ApoE e4 isoform (phenotypes e2/4, e3/4, e4/4) and as non-e4 carriers for subjects without the ApoE e4 isoform (phenotypes e2/2, e2/3, e3/3). At baseline and first follow-up, cardiovascular disease (cardiac disease and stroke) was assessed by combining self-report data, medication use and records of the general practitioners (GP) in an algorithm previously described (Bremmer et al., 2006). At second follow-up self-report data and medication records were used. Hypertension was assessed by measurement of blood pressure (≥ 160/100 mm/Hg), use of anti-hypertensive drugs or both. Diabetes mellitus was assessed by combining self-report data ($\kappa = 0.85$ (nearly perfect agreement with GP information)) (Kriegsman et al., 1996), medication use, and records of the GP. Arthritis and atherosclerosis were assessed by means of self-report ($\kappa = 0.31$ (fair agreement with GP information), and $\kappa = 0.38$ (fair agreement with GP information) respectively) (Kriegsman et al., 1996). Intake of lipid-lowering drugs and intake of non-steroidal anti-inflammatory drugs were determined by checking medication use. Depressive symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D). The CES-D is a 20-item self-report scale (range, 0-60) designed to measure depressive symptoms in the general population (Beekman et al., 1997; Radloff, 1977). BMI was calculated as weight (kg) / (height (m))^2. Alcohol consumption was assessed by asking for the number of alcoholic units consumed per week over the past year, and the number of days of the week in which alcohol was consumed, and was thereafter classified as no, middle, and high consumption

according to the NEI index (Reinhard and Rood-Bakker, 1998). Smoking status was classified as never, former, and current. Physical activity was measured by the LASA physical activity questionnaire (LAPAQ) (Stel et al., 2004). The LAPAQ is a face-to-face questionnaire in which the frequency and duration of the following activities during the past two weeks are estimated: walking outside, bicycling, light household activities, heavy household activities, and a maximum of two sport activities. In the current study these scores were converted to total time spent on physical activities in minutes per day.

Data analysis

Serum levels of tHcy, vitamin B_{12}, creatinine, CRP, ACT, IL-6 were not normally distributed, therefore ln transformations were performed. Furthermore, the MMSE score was transformed (ln (31-MMSE score)) to obtain a near-normal distribution.

Generalized estimating equations (GEE) were used to analyze longitudinal associations between tHcy, cognitive performance and rate of cognitive decline during six years of follow-up, and the possible modifying role of ACT, CRP and IL-6 (Twisk and De Vente, 2000). The dependency of repeated observations within subjects is taken into account by GEE, which is an important feature necessary for longitudinal analyses. By assuming a certain correlation structure in the repeated observations of the outcome variable, this dependency is added to the analyses. In the current study, an exchangeable correlation structure was assumed, which means that the correlation was constant between any two cognitive scores attained by one person. Another important advantage of GEE is that subjects are included regardless of missing values. Thus, subjects who were lost to follow-up after two measurements were also included in the analyses. This reduces bias that might have arisen from a differential loss to follow-up of the more cognitively impaired elderly. Subjects with cognitive data on only one measurement were excluded from the analyses, as we were interested in (the rate of) cognitive decline over time. For 76% of the subjects included in the analyses, an MMSE score was available on all three measurements, 24% had two measurement points. Data on the other cognitive tests were available for about 70% of the subjects on all three measurements, 30% had two measurement points.

First, to be able to study the association between level of cognitive functioning and tHcy, the cognitive score at baseline, three-year follow-up and six-year follow-up was the outcome measure in the longitudinal regression models. Second, to be able to determine whether tHcy was associated with the rate of cognitive decline, the interaction with time was evaluated by adding the product term between tHcy and time to the models.

The longitudinal regression models were all adjusted for time and age. All potential confounders (measured at baseline, three-year follow-up, and six-year follow-up) were added one by one to the regression model. Variables that showed a significant confounding effect on the studied associations, i.e. $\geq$ 10% change in the unstandardized regression coefficient (B) were retained in the model.

Correlations between tHcy and the inflammatory markers ACT, CRP and IL-6 were studied by means of Pearson correlation coefficients. Effect modification by ACT, CRP, and IL-6 was investigated by including product terms in the fully adjusted models. If a significant interaction was detected, separate analyses were performed to estimate the effect of tHcy for each quantile of the serum inflammatory marker (Figueiras et al., 1998). If a modifying effect was not found, the inflammatory markers were added to the analyses to study potential confounding. To study effect modification by ACT, CRP, and IL-6 on the association between tHcy and the rate of cognitive decline, a three-way interaction term (tHcy x inflammatory marker x time) was added to the models and tested for significance.

All analyses were tested at the 0.05 level of significance, except for effect modification for which a level of significance of 0.10 was tolerated, because of the multiplication of the measurement error.

Results

Table 1 shows the baseline characteristics of the total study sample.

The results show a significant negative association between level of tHcy at baseline and level of cognitive performance over the follow-up period of six years after adjustment for time and age. In particular, a negative association was found between tHcy and the MMSE score ($N = 1{,}033$, $\beta = -0.120$, $p < 0.001$), immediate recall ($N = 927$, $\beta = -0.097$, $p < 0.001$), and retention ($N = 967$, $\beta = -0.061$, $p = 0.020$) after additional adjustment for the relevant confounders. This indicates that a higher level of tHcy at baseline was significantly associated with a lower level of cognitive performance over six years of follow-up independent of relevant confounders.

Subsequently, the product term between tHcy and time was added to the fully adjusted models to study the longitudinal associations between tHcy level at baseline and rate of cognitive decline. The results show a significant negative interaction between tHcy and time in the models predicting information processing speed ($p = 0.072$) and fluid intelligence ($p = 0.033$). This indicates that a higher level of tHcy at baseline was significantly associated with a faster rate of decline in information processing speed and fluid intelligence over six years of follow-up. The results of these longitudinal analyses are shown in Figures 2 and 3.

Table 1 Baseline characteristics of subjects in the study sample		
Characteristic		Total sample (N = 1,257)
Age, years, mean (SD)		75.39 (6.56)
Men, % (N)		48.5 (610)
Education, years, mean (SD)		8.90 (3.29)
Total homocysteine, µmol/L, median (IR)		13.56 (11.10-16.90)
Vitamin B_{12}, pmol/L, median (IR)		263.00 (213.00-327.00)
Creatinine, µmol/L, median (IR)		89.00 (79.00-102.00)
ACT, % of NHP, median (IR)		157.00 (133.00-184.00)
CRP, µmol/mL, median (IR)		3.20 (1.50-6.50)
IL-6, pg/mL, median (IR)		1.90 (1.10-3.10)
Total cholesterol, mmol/L, mean (SD)		5.69 (1.03)
HDL cholesterol, mmol/L, mean (SD)		1.34 (0.42)
LDL cholesterol, mmol/L, mean (SD)		3.67 (0.95)
Triglycerides, mmol/L, mean (SD)		1.52 (0.77)
Fructosamine, µmol/L, mean (SD)		234.91 (37.81)
ApoE e4, % (N)		26.5 (332)
CES-D Depressive symptoms, mean (SD)		8.10 (7.72)
Hypertension yes, % (N)		58.1 (724)
Diabetes mellitus yes, % (N)		8.8 (111)
Cardiovascular disease possible or definite, % (N)		32.4 (407)
Rheumatic disease, % (N)		11.1 (139)
Atherosclerosis, % (N)		12.4 (156)
Use of lipid-lowering medication, % (N)		5.1 (64)
Use of anti-inflammatory medication, % (N)		29.2 (366)
Physical activity (minutes/day)		157.66 (102.79)
Body mass index, mean (SD)		26.69 (4.17)
Smoking, % (N)	No	35.6 (447)
	Former	46.0 (578)
	Current	18.4 (231)
Alcohol consumption, % (N)	No	24.5 (308)
	Middle	65.9 (828)
	High	9.6 (120)
Mini-Mental State Examination, mean (SD) (N=1,255)		26.83 (3.03)
Immediate recall, mean (SD) (N=1,232)		8.08 (2.62)
Retention, mean (SD) (N=1,229)		67.33 (26.18)
Information processing speed, mean (SD) (N=1,201)		23.13 (7.29)
Fluid intelligence, mean (SD) (N=1,199)		17.44 (4.07)

IR interquartile range; *ACT* α1-antichymotrypsin; *CRP* C-reactive protein; *IL-6* Interleukin-6; *LDL* low-density lipoprotein; *HDL* high-density lipoprotein; *ApoE* Apolipoprotein E.

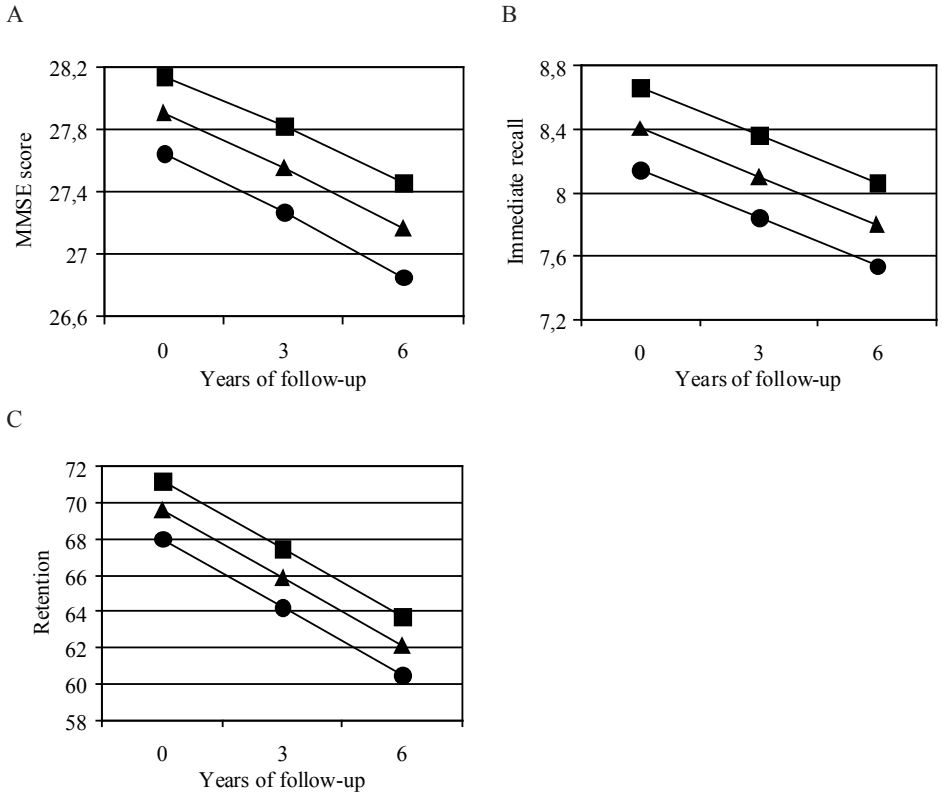

Figure 2 Six-year cognitive decline according to total homocysteine level

(A) MMSE, model adjusted for time, age, education and creatinine; (B) Immediate recall, model adjusted for time, age, education, sex, vitamin B_{12}, creatinine and HDL cholesterol; (C) Retention, model adjusted for time, age, sex and hypertension. Total homocysteine level: (■) mean - 1 SD; (▲) mean; (●) mean + 1 SD. *MMSE* Mini-Mental State Examination; *HDL* high-density lipoprotein.

The correlations between tHcy and the inflammatory markers were significant but weak. The correlation between tHcy and ACT was highest ($r = 0.156$; $p < 0.001$), with IL-6 lowest ($r = 0.082$; $p = 0.004$), and with CRP in between ($r = 0.132$; $p < 0.001$). Examination of the modifying effect of the inflammatory markers on the association between tHcy and level of cognitive functioning showed a significant interaction between tHcy and IL-6 with respect to the association with immediate recall. The negative association between higher tHcy and level of immediate recall was strongest in persons with a high level of IL-6. Also, a significant interaction was found between ACT and tHcy in the regression model predicting level of information processing speed, indicating a modifying role of ACT. In separate analyses it was shown that the negative effect of high tHcy on level of information processing speed was significant only in persons in the middle tertile of ACT. The effect of tHcy was not significant in persons in the lowest and highest tertile of ACT. In addition, the results show that the association between tHcy

and retention was significantly modified by CRP. Separate analyses show the strongest negative association between high tHcy and level of retention over time in persons in the highest tertile of CRP. The effect of tHcy was not significant in persons in the lowest and middle tertile of CRP. None of the other interaction terms reached statistical significance. Table 2 shows the results of these separate longitudinal analyses.

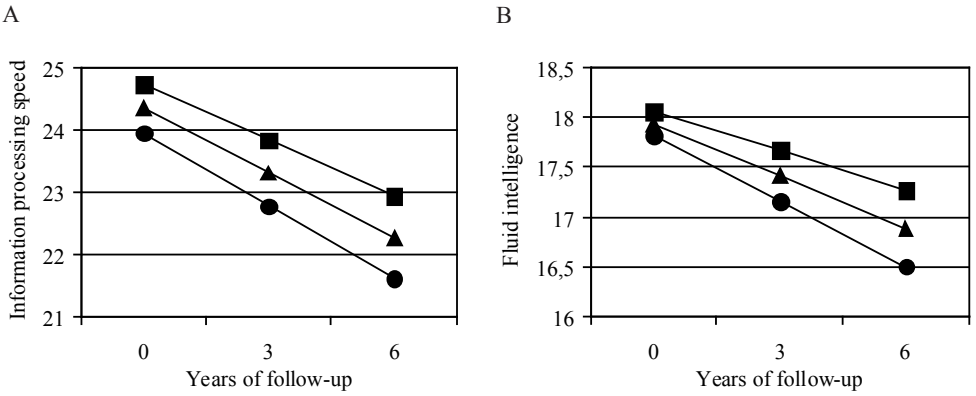

Figure 3 Six-year cognitive decline according to total homocysteine level

(A) Information processing speed, model adjusted for time, age, education, sex, vitamin B_{12}, creatinine and HDL cholesterol; (B) Fluid intelligence, model adjusted for time, age, education, sex, vitamin B_{12}, creatinine, HDL cholesterol, triglycerides, and α1-antichymotrypsin. Total homocysteine level: (■) mean - 1 SD; (▲) mean; (●) mean + 1 SD. *HDL* high-density lipoprotein.

Table 2 Association between homocysteine and cognitive performance over 6 years of follow-up by serum inflammatory marker

	IL-6, pg/mL					
	Low (0.0 – 5.0)			High (5.1 – 76.0)		
Immediate recall [a]	β [1]	p value		β [1]	p value	p interaction
(N = 914)	- .095	***	.001	- .124	* .016	.061
	Tertile of CRP, µmol/mL					
	I (0.2 – 1.9)		II (2.0 – 5.2)		III (5.3 – 171.0)	
Retention [b]	β [1]	p value	β [1]	p value	β [1]	p value	p interaction
(N = 949)	- .054	.185	- .055	.244	- .089	* .050	.088
	Tertile of ACT, % NHP					
Information	I (24-140)		II (141-172)		III (173-437)	
processing speed [c]	β [1]	p value	β [1]	p value	β [1]	p value	p interaction
(N = 893)	- .090	.054	- .110	* .043	- .0075	.883	.090

[a] Adjusted for time, age, education, sex, vitamin B_{12}, creatinine, and HDL cholesterol; [b] Adjusted for time, age, sex, and hypertension; [c] Adjusted for time, age, education, sex, vitamin B_{12}, creatinine, and HDL cholesterol; [1] Standardized regression coefficients. *IL-6* Interleukin-6. *CRP* C-reactive protein; *ACT* α1-antichymotrypsin; *HDL* high-density lipoprotein. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Finally, the modifying effect of the inflammatory markers on the association between tHcy and rate of cognitive decline was studied by including a three-way interaction between tHcy, the inflammatory markers ACT, CRP and IL-6 and time in the fully adjusted models. The results show a significant negative interaction between tHcy, ACT and time ($p = 0.065$) and tHcy, CRP and time ($p = 0.041$) in the fully adjusted models predicting performance on the coding task. This indicates that ACT and CRP significantly modify the association between tHcy and rate of decline in information processing speed. Separate longitudinal analyses show a significant negative interaction between tHcy and time in the middle tertile of ACT ($p = 0.016$), and both the lowest ($p = 0.059$) and middle ($p = 0.097$) tertile of CRP. The results indicate that a higher tHcy level was significantly associated with a faster rate of decline in information processing speed, only in the middle tertile of ACT, and both in the lower and middle tertile of CRP.

Discussion

The present longitudinal population-based study showed that a higher level of tHcy was an independent predictor of a lower level of general cognitive functioning, immediate recall, retention, information processing speed and fluid intelligence over six years of follow up. In addition, a higher level of tHcy was an independent predictor of a faster rate of decline in information processing speed and fluid intelligence after proper adjustment for confounders. This indicates that in older persons a higher level of tHcy is a marker of lower cognitive functioning over time, independent of other important risk factors such as age, education, vascular risk factors, vitamin B_{12} levels, and renal function, and also an independent predictor of a faster rate of decline in information processing speed and fluid intelligence.

The conclusion drawn in the present study is in line with the results from several other prospective studies focusing on the association between tHcy and change in cognitive functioning (Dufouil et al., 2003; Garcia et al., 2004; McCaddon et al., 2001; Tucker et al., 2005). Furthermore, several studies found a higher risk for dementia in persons with higher tHcy levels (Haan et al., 2007; Ravaglia et al., 2007a; Seshadri et al., 2002), which is in line with the results from the present study. The Maastricht Aging Study (MAAS) found that elevated tHcy concentrations were associated with prolonged lower cognitive performance. However, they did not observe a significant association between tHcy and cognitive change over six years of follow-up. The authors suggest that this may be due to lack of inter-individual differences in cognitive change (Teunissen et al., 2003). In the Leiden 85-Plus Study it was concluded that in old age increased concentrations of tHcy can identify individuals with cognitive impairment at baseline, but do not predict the rate of cognitive decline (Mooijaart et al., 2005). The latter is in contrast with the results from the current study and might be explained by the differences in age between the studies (85+ compared to 65+ in the current study). Other longitudinal studies have failed to find any association between tHcy and cognitive performance or risk for AD (Kado et al., 2005; Kalmijn et al., 1999; Luchsinger et al., 2004). This could be due to insufficient power (Kalmijn et al., 1999; Luchsinger et al., 2004), and differences in characteristics of the study sample (i.e.

lower tHcy levels, and inclusion of only high functioning persons in comparison to the present study) (Kado et al., 2005).

Studying the interaction between tHcy and the inflammatory markers ACT, CRP and IL-6, it was shown that inflammation seems to enhance the negative effect of high levels of tHcy on cognitive functioning over six years of follow-up in some cases. Some suggestions for potential underlying mechanisms can be made. First, the association between hyperhomocysteinemia and inflammation may promote the development and progression of atherosclerotic plaques (Gunstad et al., 2006; Papatheodorou and Weiss, 2007). In turn, increasing evidence suggests a link between AD and atherosclerosis in older persons (Newman et al., 2005; Van Oijen et al., 2007). In addition, evidence from both in vitro and in vivo studies suggest that tHcy is related to a pro-inflammatory response, while at the same time Hcy levels may be influenced by active inflammatory processes (Gori et al., 2007). In the present study we were not able to fully exclude the mediating influence of subclinical atherosclerosis, although we adjusted for important risk factors for atherosclerosis and (sub)clinical cardiovascular diseases, such as the metabolic syndrome. Second, Lazzerini et al. (2007) suggests a bi-directional relationship between hyperhomocysteinemia and immuno-inflammatory activation in the course of autoimmune diseases, in which immune activation and inflammation may contribute to hyperhomocysteinemia and vice versa. Among other mechanisms, it has been proposed that the decline in cognitive functions and neurogenesis throughout the progression of AD may also be affected by autoimmune mechanisms operating in the periphery and in the brain (Baron et al., 2007). In contrast, studies investigating the effect of lowering tHcy levels by means of folic acid supplementation have shown no effect on plasma concentrations of inflammatory markers (Durga et al., 2005; Klerk et al., 2005). Therefore, it has also been suggested that the associations between hyperhomocysteinemia and inflammatory markers might be a non-specific reflection of poor health and comorbidity (Ravaglia et al., 2004). The fact that our finding of a modifying effect of inflammation on tHcy was not a consistent one, supports the latter explanation.

The major strength of the current study is that longitudinal data from a large population-based study were used. Several important cognitive functions sensitive to aging were studied and both level of cognitive functioning over time and rate of cognitive decline were used as outcome measures. To our knowledge, the interaction between tHcy and ACT, CRP and IL-6 on cognitive functioning over time and cognitive decline has not been studied previously. The results from the present study suggest that future research should further investigate the modifying role of the inflammatory markers.

However, some possible limitations should be discussed. Level of tHcy as well as the other biological markers were only determined once, at baseline, while data on cognitive function were available at baseline as well as follow-up after three years and six years. This could lead to regression dilution and thus an underestimation of the importance of tHcy as a risk factor (Clarke et al., 2001). Furthermore, the most frail and poorest functioning persons were lost to follow-up. The persons lost to follow-up had lower scores on the cognitive tasks and

higher tHcy levels compared to those who remained in the study. The use of the longitudinal data-analysis technique GEE has partly resolved the bias caused by loss to follow-up, however an underestimation of the found associations may be likely. In addition, folic acid of which a deficiency could potentially lead to elevated levels of tHcy (Selhub and Miller, 1992), and methylmalonic acid (MMA) which is regarded by many, but not all authors as an early and specific indicator of vitamin B_{12} deficiency (Klee, 2000), were both only determined in small, separate subsamples of the total study sample. Therefore, the influence of levels of both folic acid and MMA on the associations studied could not be taken into account so that we were not able to exclude the possibility that folic acid and/or MMA affect the pathway by which tHcy is associated with cognitive performance. In addition, since levels of vitamin B_6, which is also an important determinant of plasma tHcy (Selhub and Miller, 1992), were not determined in the present study sample, the possible mediating or confounding influence of vitamin B_6 on the associations and interactions studied could not be taken into account. Finally, the issue of reverse causality needs to be addressed. Although the use of a longitudinal study with six years of follow-up instead of a cross-sectional study gave us the opportunity to study change in cognitive functioning over time, the possibility that dementia pathology led to increased tHcy and inflammation could not be fully dismissed. A formal dementia diagnosis satisfying the criteria as posed by the DSM-IV (American Psychiatric Association, 1994) or the NINCDS-ADRDA (McKhann et al., 1984) could not be made with LASA data. However, subjects showing persistent cognitive decline were identified by the use of repeated measurements of the MMSE and the IQCODE. Persistent cognitive decline was defined by clinically significant cognitive decline over at least three years of follow-up, satisfying the rather strict criterion of more than two standard deviations below the average cognitive decline observed in the total study sample, and continued decline during the subsequent three years. Therefore, most of the subjects identified with persistent cognitive decline are likely to have developed dementia (Van den Kommer et al., 2008). Within our current study sample only one person was identified with persistent cognitive decline at the time of blood sampling (baseline). Therefore, the danger of reverse causality was reduced substantially and exclusion of demented subjects at baseline or separate analysis were not considered necessary.

In sum, the present longitudinal study showed that a higher tHcy level was an independent marker of lower cognitive functioning and a faster rate of decline in information processing speed and fluid intelligence over time. Furthermore, the findings implicate that the inflammatory markers IL-6, CRP and ACT may play a modifying role with respect to the associations between higher tHcy and prolonged lower cognitive functioning and rate of cognitive decline. The results suggest that a combination of both risk factors may be used as a marker predictive of cognitive impairment.

Conflict of interest

None.

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