의학석사 학위논문

Effect of Homocysteine on Hippocampal Atrophy Independent of Cerebral Amyloid Deposition and Vascular Burden in normal aging, MCI and AD

정상노인, 경도인지장애, 알츠하이머 병에서 뇌 아밀로이드 축적, 혈관성 위험과는 독립적인 해마 위축에 대한 호모시스테인의 영향 연구

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최 영 민

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By

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ABSTRACT

Effect of Homocysteine on Hippocampal Atrophy Independent of Cerebral Amyloid Deposition and Vascular Burden in Normal aging, MCI and AD

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Objectives: To clarify whether homocysteine has any independent effect, not mediated by cerebral beta amyloid protein (Aβ) deposition and vascular burden, on whole brain or hippocampal atrophy in elderly individuals with normal cognition, mild cognitive impairment (MCI) and Alzheimer's disease (AD).

Methods: Fourteen cognitively normal, 19 MCI, and 24 AD individuals were included. All subjects received three-dimensional volumetric MRI, Pittsburgh Compound B - positron emission tomography and comprehensive clinical evaluation including vascular burden assessment for diabetes, hypertension, dyslipidemia, coronary artery disease, stroke and transient ischemic attack. Blood homocysteine, vitamin B12, and folate levels were also measured.

Results: Multiple linear regression analyses showed that plasma total homocysteine level was significantly associated with hippocampal atrophy even after controlling the degree of global cerebral Aβ deposition and vascular burden as well as other potential confounders including age, gender, education and apolipoprotein E ε4 genotype. In contrast, plasma total homocysteine level did not show any significant association with whole brain volume.

Conclusions: Our finding of the independent negative association between plasma homocysteine and hippocampal volume suggests that homocysteine has a direct adverse effect, not mediated by cerebral Aβ deposition and vascular burden, on the hippocampus.

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Keywords: homocysteine, hippocampus, amyloid deposition, vascular burden, Alzheimer's disease, mild cognitive impairment

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INTRODUCTION

Homocysteine is a sulfur amino acid involved in the metabolism of methionine, a process dependent on the vitamin B_6, cobalamin and folic acid. Homocysteine has been proposed to be one of the risk factors for cardiovascular disease,[1] carotid stenosis[2] and stroke.[3] A series of the studies also suggested that the elevation of plasma total homocysteine (tHcy) increases the risk of cognitive decline, dementia and Alzheimer's disease (AD).[4-7]

An association between plasma tHcy and brain atrophy has also been reported. Elevation of plasma tHcy level was associated with more rapid atrophy of the medical temporal lobe,[8] and smaller hippocampus size in AD patients.[9] The rate of brain atrophy in the elderly with mild cognitive impairment (MCI) was slowed by the treatment with homocysteine lowering B vitamins.[10] Even in healthy old people, plasma tHcy levels were related to small hippocampal width[11] and ventricular dilatation.[12]

The mechanism of pathogenetic linkage between homocysteine and brain atrophy is, however, still unclear, although several possibilities have been suggested.[13] Homocysteine may cause brain atrophy through potentiating beta amyloid protein (Aβ)-induced neurodegeneration in the pathophysiological process of AD.[14, 15] As Aβ deposition starts several decades before the onset of evident cognitive deficits,[16] this possibility could not be excluded even for the association between plasma tHcy and brain atrophy in healthy elderly people.[9, 11, 12] In addition, given that elevated plasma tHcy is known to be independent risk factor for vascular disease[17] and brain atrophy was also observed in vascular dementia,[18] homocysteine may influence brain volume through

vascular pathology. In contrast, the possibility that homocysteine has direct neurotoxic effect, not mediated by Aβ and vascular pathology, was also suggested through preclinical studies.[19-21]

We aimed to clarify whether homocysteine has any independent effect, not mediated by cerebral Aβ deposition and vascular burden, on whole brain or hippocampal atrophy in elderly individuals with normal cognition, MCI and AD. We applied ^{11}C-labeled Pittsburgh Compound B (^{11}C-PiB) PET imaging[22] to quantify the cerebral Aβ deposition.

METHODS

Subjects

Nineteen mild cognitive impairment (MCI) and 24 AD patients were recruited from the Dementia and Age-Associated Cognitive Decline Clinic of the Seoul National University Hospital. The AD patients met the criteria for dementia of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) and criteria of probable AD of the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorder Association (NINCDS-ADRDA). Individuals with MCI met current consensus criteria[23]: (a) memory complaint corroborated by an informant, (b) objective memory impairment for age, education and gender, (c) essentially preserved general cognitive function, (d) largely intact functional activities, (e) not demented. Fourteen cognitively normal (CN) elderly subjects with an overall CDR of 0 were also selected from a pool of elderly volunteers. All subjects were included after a standardized clinical assessment, as described below.

The following exclusion criteria were applied to all subjects: any present serious medical, psychiatric, or neurological disorder that could affect mental function; evidence of focal brain lesions on MRI including lacunes and white matter hyperintensity lesions of grade 2 or more by Fazeka scale[24]; the presence of severe behavioral or communication problems that would make a clinical or imaging examination difficult; presence of severe renal insufficiency defined as serum creatinine level above 1.5 mg/dL; and the absence of a reliable informant.

The Institutional Review Board of Seoul National University Hospital, approved the study protocol and informed consent was obtained from all study subjects and their relatives.

Clinical and neuropsychological assessment

All subjects were examined by neuropsychiatrists with advanced training in dementia research according to the protocol of the Korean version of the Consortium to Establish a Registry for AD (CERAD-K).[25] Psychiatric, general physical and neurological examinations were also included. A panel consisting four neuropsychiatrists with expertise in dementia research made clinical decisions, including the assignment of Clinical Dementia Rating (CDR).

The presence or absence of six cerebrovascular risk factors including stroke, diabetes, dyslipidemia, transient ischemic attack, hypertension, and coronary artery disease was systematically assessed from subject and informant histories as well as review of pertinent medical records. To calculate an overall measure of cerebrovascular burden, we created a composite score that was the sum of the factors present ranging from 0 to 6[26] and reported as a percentage.

Eight neuropsychological tests in the Consortium to Establish a Registry for Alzheimer's Disease neuropsychological battery including Verbal Fluency, 15-item Boston Naming test, Mini-Mental State Exam, Word List Memory, Word List Recall, Word List Recognition, Constructional Praxis, and Construction Recall test and Stroop Color-Word test were also conducted by experienced clinical psychologists.

MRI image acquisition and analysis

MRI acquisition

MRI was performed using a whole-body 3T system (Signa VH/i; General Electric, Milwaukee, WI, USA). A dual spin-echo echo-planar imaging (EPI) sequence was used to acquire DTI images. MR images with 25 non-collinear diffusion gradients and without diffusion gradient were acquired (TR = 10000 ms, TE = 77.1 ms, B-factor = 1000 s/mm2, matrix = 128×128, slice thickness/gap = 3.5/0 mm, FOV= 240 mm, slice number = 38). A three-dimensional T1-weighted spoiled gradient recalled echo (SPGR) sequence was obtained for volumetric tracing and anatomical localization (TR = 22.0 ms, TE = 4.0 ms, slice thickness/gap= 1.4/0 mm, matrix = 256×192, FOV= 240 mm, Flip angle = 40º). Additionally, fluid-attenuated inversion recovery (FLAIR) and T2-weighted images were also obtained for qualitative clinical reading.

Volumetric measurement

Hippocampal volume

The anatomical boundaries of hippocampus were traced manually on T1-weighted images using Analyze AVW 5.0 (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN) and all traces were drawn blind to diagnosis, sex, or subject demographics. Sagittal SPGR sequence Images were realigned to a standard orientation and reformatted using sinc interpolation to a 0.94-mm slice thickness in the axial plane. The standard alignment was based on the interhemispheric fissure, the lenses of both eyes, and the line connecting the anterior and posterior commissures in the sagittal plane.

Tracing began with the generation of the auxiliary guideline traces on the

sagittal plane, and included the subiculum, Ammon's horn (hippocampus proper), and the dentate gyrus.[27] And the regions of interest were finally defined on the coronal plane. The borders of the hippocampus were defined as follows. For the hippocampal had, medial/lateral borders are the ambient gyrus or entorhinal sulcus/the temporal horn of the lateral ventricle and dorsal/ventral borders are the alveous/the white matter of the temporal lobe or the subiculum. For the body of the hippocampus, medial/lateral border is the ambient cistern and the crus cerebri/the temporal stem and inferior horn of the lateral ventricle and dorsal/ventral borders are the CSF of the lateral ventricle/the white matter of the temporal lobe. For the tail of the hippocampus, medial/lateral borders are the CSF of the atrium of the lateral ventricles/the ascending crus of the fornix and dorsal/ventral borders are the pulvinar of the thalamus/the white matter of the temporal lobe. To determine the reliability of volumetric measurements, the same rater, unaware of previous readings, repeated volume tracing on 10 randomly selected subjects. Reliability, expressed as intraclass correlation coefficients, was 0.97 for the hippocampus. Mean volume of the left and right hippocampus was used for further analyses.

Whole brain and total intracranial volume

Images were processed using the standard Montreal Neurological Institute (MNI) anatomical pipeline. The native T1 images were normalized into a standardized stereotaxic space using a linear transformation and corrected for intensity non-uniformity.[28, 29] Each subject's brain, which was transformed and corrected, was classified into WM, GM, CSF and background using a 3-D stereotaxic brain mask and the INSECT (Intensity-Normalized Stereotaxic

Environment for Classification of Tissues) algorithm.[30] Whole brain volume (WBV) was calculated as sum of WM and GM volume which was inverse-transformed to native space. We calculated the total intracranial volume (ICV) by measuring the volume of voxels within the brain mask. The brain mask was generated using the brain extraction tool.[31] WBV and hippocampal volume was normalized by ICV to correct for possible differences in head size.

^{11}C-PiB PET image acquisition and analysis

^{11}C-PiB PET was performed using the ECAT EXACT47 scanner (Siemens-CTI, Knoxville, TN), which had an intrinsic resolution of 5.2-mm full width at half maximum. Before administering ^{11}C-PiB, a 10-minutetransmission scanning, was performed, using rotating three germanium-68 rod sources to correct the attenuation. Sixty minutes after the intravenous injection of 370 MBq ^{11}C-PiB, three 10-minute frames of data acquisition were started and later summed into a single frame (60–90 minutes). All the data were reconstructed in 123 × 128 × 47 matrix with a pixel size of 2.57 × 2.57 × 3.75 mm using the filtered back projection method with Shepp-Logan filter (cutoff =0.35 cycle/pixel), and reconstructed images were corrected for attenuation and rearranged onto transaxial, sagittal, and coronal images.

Image preprocessing for statistical analyses was performed using statistical parametric mapping2 (SPM2, Wellcome Department of Cognitive Neurology, London, United Kingdom) implanted in the Matlab (Mathworks, Natick, MA). ^{11}C-PiB PET data of each subject was co-registered to individual volumetric magnetic resonance image and then automatically spatially normalized into the standard MNI template in SPM2 using transformation parameters derived from the normalization of individual magnetic resonance image to the

template. All normalized images were reformatted with a voxel size of 2×2×2 mm. For quantitative normalization of cerebral ^{11}C-PiB uptake values, the cerebellum, which is relatively free of Aβ deposition, was used as a reference region[32] and ^{11}C-PiB retention maps as region-to-cerebellar ratio were generated by dividing regional uptake values by the individual mean cerebellar uptake values in the same images.

The automatic anatomic labeling algorithm[33] and a region combining method[34] were applied to set region-of-interest (ROI) to characterize ^{11}C-PiB retention in the frontal, lateral parietal, posterior cingulate-precuneus (PC-PRC), lateral temporal and occipital, and basal ganglia regions, where prominent ^{11}C-PiB retention was reported.[22] The subregions included in each ROI were as follows: the frontal ROI included bilateral frontal except precentral gyrus, anterior cingulate, middle cingulate, and insular cortex regions (automatic anatomic labeling region No. 3-34); the lateral parietal ROI included all bilateral lateral parietal regions except postcentral gyrus (59–65); the PC-PRC ROI included bilateral posterior cingulate (35–36) and precuneus (67–68); the lateral temporal ROI included all bilateral temporal regions except medial temporal structures (79–90); the occipital ROI included all bilateral occipital regions (43–56); the basal ganglia ROI included bilateral caudate, putamen, and globus pallidus regions (71–76). A global cortical ROI consisting of frontal, lateral parietal, PC-PRC, lateral temporal and BG ROIs was also defined. For each ROI, mean ^{11}C-PiB value was calculated by averaging ^{11}C-PiB retention values for all voxels within ROI. Global amyloid burden was defined as a mean ^{11}C-PiB retention value of the global cortical ROI. The image was classified as PiB-positive if mean ^{11}C-PiB retention value was over 1.4 in one of the following ROIs: frontal, lateral temporal,

lateral parietal, PC-PRC and BG.[34]

Blood sample collection and analysis

Plasma tHcy concentrations were determined by using chemiluminescent microparticle immunoassay technology (ARCHITECT homocysteine reagent kit, Abbott, IL, USA). Serum concentrations of vitamin B12 and folate were also measured with a radioassay kit with the use of ^{57}Co and ^{125}I, respectively (SimulTRAC-SNB radioassay kit, MP biomedicals, NY, USA). Serum creatinine levels were determined using the Jaffe reaction method (ADVIA-1650 kit; Bayer Corp., Pittsburgh, Pennsylvania, USA).

Apolipoproteine E genotyping was performed according to previously described methods.[35] ApoE ε4 carrier was defined as subject who had at least one ApoE ε4 allele.

Statistical analysis

Group differences of subjects' characteristics were examined by ANOVA with post hoc contrasts using Tukey's method. Categorical variables were compared using chi-square test or Fisher's exact test. The relationships of WBV and hippocampal volume with related variables including age, education, plasma tHcy, vitamin B_{12}, folate level, vascular risk score and amyloid burden were investigated by using Pearson's correlation analysis. The associations between plasma tHcy level and related variables including age, plasma vitamin $B_{12,}$ folate level, serum creatinine, body mass index, vascular risk score and global amyloid burden were also investigated by using the same analysis. Pearson's partial correlation coefficients were calculated to control the influence of age and gender on the associations between plasma tHcy level

and hippocampal volume and WBV. Multiple linear regression analyses were performed to explore the associations between plasma tHcy level and WBV and hippocampal volume. Effects of brain volume related variables including age, gender, education, ApoE ε4 genotype allele, vascular risk score and amyloid burden were controlled as covariates in the regression model. The same multiple linear regression analyses were also done only for ^{11}C-PiB negative subjects. P value < 0.05 was considered significant. All statistical analyses were performed using SPSS software (version 18.0 for windows; SPSS Inc., Chicago, IL, USA)

RESULTS

Subject characteristics

Demographic and clinical characteristics of the subjects are summarized in table 1. Subject age ranged from 56 to 89 years with mean of 71.3 years and the proportion of female was 75%. There was no significant age difference between CN, MCI and AD, but gender showed significant group difference. Mean duration of education was 8.9 years and AD group had significantly lower education compared to CN group. AD group had smaller hippocampal volume and more severe global amyloid burden compared to CN and MCI group. Significant group differences were found in the ApoE ε4 genotype, CDR and CDR SOB, but not in the whole brain volume and vascular risk score. CN group showed significantly lower body mass index compared to MCI group. Mean plasma tHcy level for overall subjects was 9.14 μmol/L (SD 2.83) and 3 participants (5%) had levels above normal range (>15μmol/L). Plasma folate and vitamin B_{12} levels of all subjects were within normal range and no subjects had pathological deficiency of folate (<3μg/mL) or vitamin B_{12} (<200pg/mL). No significant group differences were found in the plasma tHcy, vitamin B_{12}, folate and serum creatinine levels.

Table 1. Subjects characteristics

	All (n=57)	CN (n=14)	MCI (n=19)	AD (n=24)	P value
Age, year	71.33± 7.61	72.64± 5.49	73.00± 7.39	69.25± 8.56	0.212
Gender, n (M/F)	14/43	8/6	3/16	3/21	0.008
Education, year	8.93±4.82	11.86±4.11	8.37±4.48	7.67±4.92	0.026*
ApoE ε4 carrier, n (%)	23 (40.4)	1 (7.1)	9 (47.4)	13 (54.2)	0.013
PiB positive, n (%)	29 (50.9)	1 (7.1)	9 (47.4)	19 (79.2)	<0.001
CDR					<0.001
0	14	14	0	0	
0.5	33	0	19	14	
≥ 1	10	0	0	10	
CDR SOB	2.39±2.35	0.00±0.00	1.68±0.48	4.33±2.33	<0.001*†‡
Hippocampal vol., %ICV	0.153±0.033	0.181±0.019	0.162±0.022	0.129±0.030	<0.001*†
Whole brain vol., %ICV	77.93±0.04	78.16±0.04	79.19±0.03	76.78±0.04	0.097
Global amyloid burden	1.33±0.33	1.05±0.22	1.26±0.24	1.56±0.28	<0.001*†
Vascular risk score, %	1.04±0.76	1.00±0.88	1.11±0.66	1.00±0.78	0.888
Body mass index, kg/㎡	23.68±3.12	22.33±2.45	25.03±3.14	23.43±3.17	0.044‡
Serum Creatinine, mg/dL	0.96±0.11	1.00±0.17	0.96±0.10	0.95±0.11	0.568
Homocysteine, μmol/L	9.14±2.83	7.79±1.53	9.24±2.04	9.83±3.64	0.097
$VitB_{12}$, pg/mL	798.14±356.07	874.1±330.32	755.79±409.33	787.38±332.86	0.637
Folate, μg/mL	12.38±12.01	14.61±14.54	13.88±12.24	9.88±10.16	0.410

CN, cognitively normal; MCI, Mild cognitive impairment; AD, Alzheimer's disease; PiB, 11C-labeled Pittsburgh Compound B; CDR, clinical dementia rating; SOB, sum of boxes; ICV, intracranial volume.
Data are presented as mean±SD. Hippocampal volume is the mean volume of the left and right hippocampus. Comparison of three diagnostic groups was done by ANOVA with post hoc contrasts using Tukey's methods : *CN vs AD, †MCI vs AD, ‡CN vs MCI. Chi-square test was performed to compare the number of ApoE ε4 carriers and PiB positive subjects between the groups. Fisher's exact test was used to compare gender and CDR between the groups.

Simple correlations between brain volume and related variables

Results of Pearson's correlation analysis between brain volume and other variables including age, education, plasma tHcy, vitamin B_{12}, folate level, vascular risk score and amyloid burden for overall subjects are presented in table 2. Age and plasma tHcy level showed significant negative correlation with WBV (r=-0.289, p=0.029; r=-0.303, p=0.022 respectively). Plasma folate level showed marginally significant correlation with WBV (r=0.242, p=0.070). Plasma tHcy level and global amyloid burden showed significant negative association with hippcocampal volume (r=-0.315, p=0.017; r=-0.427, p<0.001, respectively).

Table 2. Correlations between brain volume and related factors for overall subjects (n=57)

	Whole brain volume (% ICV)		Hippocampal volume (% ICV)	
	r	P value	r	P value
Age	-0.289	0.029	0.008	0.950
Education	0.127	0.347	0.165	0.219
Log-tHcy	-0.303	0.022	-0.315	0.017
Log-VitB_{12}	-0.144	0.286	0.151	0.262
Log-Folate	0.242	0.070	0.203	0.130
Vascular risk score	0.077	0.567	0.016	0.906
Global amyloid burden	-0.038	0.782	-0.427	<0.001

ICV, intracranial volume.
Log-tHcy, Log-VitB12, Log-Folate: natural log-transformed plasma total homocysteine, vit B12, folate to achieve normal distribution.

Simple correlations between homocysteine and related variables

The results of Pearson's correlation analysis between homocysteine and other variables including age, plasma vitamin $B_{12,}$ folate level, serum creatinine and body mass index are presented in table 3. Plasma folate level was negatively correlated and serum creatinine was positively correlated with plasma tHcy (r=-0.309, p=0.019; r=0.347, p=0.014, respectively). Vitamin B_{12} level showed marginally significant correlation with plasma tHcy (r=-0.249, p=0.062). Plasma tHcy was not significantly correlated with vascular risk score and global amyloid burden.

Table 3. Correlations between homocysteine and related variables for overall subjects (n=57)

	r	P value
Age	0.195	0.145
Log-VitB_{12}	-0.249	0.062
Log-Folate	-0.309	0.019
Serum creatinine	0.347	0.014
Body mass index	-0.037	0.788
Vascular risk score	0.056	0.679
Global amyloid burden	-0.066	0.626

Log-tHcy: natural log-transformed plasma total homocysteine to achieve normal distribution.

Partial correlations between homocysteine and brain volume

Plasma total homocysteine level showed marginally significant negative correlation with WBV after controlling age and gender (R^2=0.067, P=0.056) (Fig 1, A). In contrast, negative correlation between plasma tHcy and hippocampal volume remained significant even after controlling age and gender (R^2 =0.112, P =0.013) (Fig 1, B).

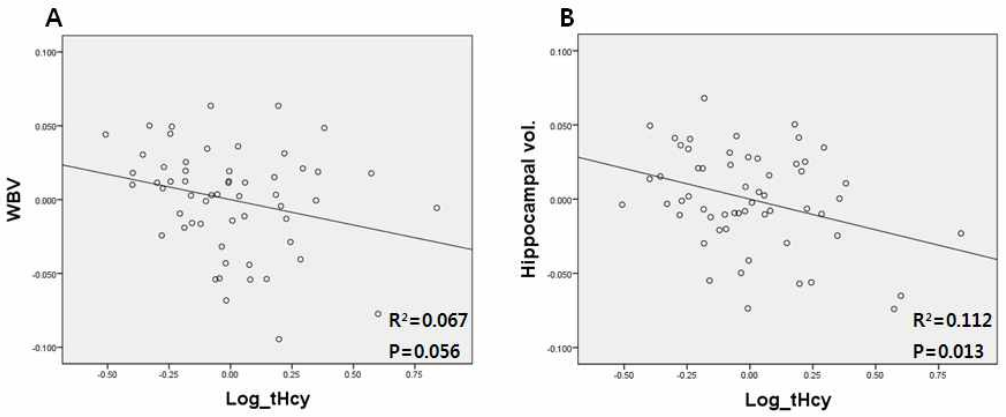

Figure1. Partial regression plots for the relationship between brain volume and plasma tHcy level for overall subjects.
Residuals are plotted for each variable to adjust for the effects of age, gender. Log-tHcy: plasma total homocysteine was natural log-transformed to achieve normal distribution. Whole brain volume and hippocampal volume were corrected for intracranial volume (%).

Multiple regression analysis between homocysteine and brain volume for overall subjects

First, we tested a multiple linear regression model with age, gender, education, APOE ε4 genotype, vascular risk score and global amyloid burden as covariates to investigate independent effect of homocysteine on WBV (Table 4, A). Plasma tHcy level did not show any significant association with WBV after controlling the covariates.

Next, we investigated independent effect of homocysteine on hippocampal volume (Table 4, B). Plasma tHcy level was significantly associated with hippocampal atrophy even after controlling age, gender, education, APOE ε4 genotype, vascular risk score and amyloid burden (β=-0.348, p=0.014). In addition, global amyloid burden also showed significant independent effect on hippocampal atrophy (β=-0.521, p<0.001), while vascular risk score did not show any significant association with hippocampal volume.

Table 4. Results from multiple regression analysis for overall subjects (n=57)

Covariates	B (SE)	β	p
A. Dependent variable: whole brain volume			
Age	-0.002 (0.001)	-0.352	0.016
Gender	-0.006 (0.013)	-0.448	0.632
Education	0.0005 (0.001)	0.070	0.660
ApoE ε4 carrier	0.018(0.010)	0.246	0.080
Vascular risk score	0.006 (0.006)	0.123	0.359
Amyloid burden	-0.029 (0.017)	-0.264	0.092
Log-tHcy	-0.028(0.019)	-0.209	0.153
B. Dependent variable: hippocampal volume			
Age	-0.001 (0.001)	-0.124	0.356
Gender	0.002 (0.011)	0.032	0.823
Education	-0.0004 (0.001)	-0.067	0.659
ApoE ε4 carrier	0.003 (0.009)	0.043	0.743
Vascular risk score	-0.002 (0.005)	-0.050	0.691
Amyloid burden	-0.052 (0.015)	-0.521	<0.001
Log-tHcy	-0.042 (0.016)	-0.348	0.014

B: Regression coefficient, SE: Standard error, β: Standardized beta.
Log-tHcy: natural log-transformed plasma total homocysteine to achieve normal distribution. Whole brain volume and hippocampal volume were corrected for intracranial volume (%).

Multiple regression analysis between homocysteine and brain volume only for ^{11}C-PiB negative subjects

Multiple regression analyses were also performed for 28 subjects classified as ^{11}C-PiB negative (Table 5) controlling age, gender, education, ApoE ε4 genotype and vascular risk score. Plasma tHcy level was significantly associated with hippocampal atrophy independent of vascular risk score as well as age, gender, education and ApoE ε4 genotype (β=-0.661, p=0.008), but not with WBV.

Table 5. Associations of covariates with brain volume in ^{11}C-PiB negative subjects (n=28)

Covariates	B (SE)	β	p
A. Dependent variable: whole brain volume			
Age	-0.004(0.001)	-0.623	0.003
Gender	0.001(0.017)	0.016	0.938
Education	-0.0009 (0.002)	-0.108	0.688
ApoE ε4 carrier	0.017 (0.018)	0.173	0.342
Vascular risk score	0.004 (0.009)	0.077	0.659
Log-tHcy	-0.008 (0.034)	-0.058	0.812
B. Dependent variable: hippocampal volume			
Age	-0.001 (0.001)	-0.248	0.174
Gender	0.017 (0.011)	0.282	0.159
Education	-0.002 (0.001)	-0.421	0.105
ApoE ε4 carrier	0.005 (0.012)	0.074	0.659
Vascular risk score	-0.003 (0.006)	-0.080	0.623
Log-tHcy	-0.067 (0.023)	-0.662	0.008

B: Regression coefficient, SE: Standard error, β: Standardized beta.
Log-tHcy: natural log-transformed plasma total homocysteine to achieve normal distribution. Whole brain volume and hippocampal volume were corrected for intracranial volume (%).

DICUSSION

Our investigation revealed that plasma tHcy level was closely associated with hippocampal atrophy even after controlling the effects of global amyloid burden and vascular risks as well as age, gender, education, ApoE ε4 genotype in elderly people including normal aging, MCI and AD individuals. We also found a similar independent association between plasma tHcy and hippocampal volume for the subgroup of subjects classified as ^{11}C-PiB negative, i.e., subjects with no or very low cerebral amyloid burden and so probably not related to AD pathological process. To the best of our knowledge, this is the first study that showed the indepdent effect of homocysteine, not mediated by cerebral Aβ deposition and vascular burden, on hippocampal atrophy in elderly individuals. Although there were several studies on the relationship between plasma tHcy and brain atrophy in cognitively healthy elderly people,[9, 11, 12] the confounding effect of amyloid burden was unlikely to have been fully excluded as Aβ deposition begins several decades before the onset of evident cognitive decline.[16]

Our finding of the independent negative relationship of homocysteine with hippocampal volume supports the possibility of direct neurotoxic effect of homocysteine in human brain. Several preclinical studies suggested the direct neurotoxicities of homocysteine and their potential mechanisms. It was reported that homocysteine increased oxidative stress by generating hydrogen peroxide and other reactive oxygen species.[19] In addition, homocysteine may not only overstimulate N-methyl-D-aspartate (NMDA) receptors that results in brain excitotoxicity,[20] but also impair transmethylation and promotes

apoptosis through DNA damage.[21] These potential pathogenetic mechanisms implicated by preclinical investigations may underlie the independent negative influence of homocysteine on hippocampal volume observed in the current study.

In contrast to the results on hippocampal volume, we could not find independent associations between plasma tHcy and WBV. However, before controlling the effect of potential confounders including global amyloid burden and vascular risk scores, there was a signficant correlation between tHcy and WBV. Given these findings together, it is likely that the effect of Homocysteine on WBV is mainly mediated by other potential confounders, and direct negative effect of homocysteine is relatively very small. Previous studies[9,36] suggested the level-dependent negative effect of homocysteine on brain volume, based on a prominent homocysteine -brain atropy association in the group with pathologically elevated plasma tHcy. Considering that plasma tHcy levels in most of our subjects were within normal range, the size of direct neurotoxic effect of homocysteine on overall brain is possibly too small to be detected, while the effect on the hippcampus, known as a particularly vulnerable brain structure to various harmful insults,[37] could be found.

Although homocysteine is a well-known, independent risk factor for cerebrovascular disease,[17] and cerebrovascular burden was reported to contribute to brain atrophy,[38] we did not find any significant relationship between plasma tHcy and vascular risk scores, or between vascular risk scores and brain atrophy. This is probably because most of our subjects had very low vascular burden. Mean vascular risk score was low and the variation of the score was also very small. As we mainly aimed to investigate the independent effect of homocysteine, not mediated by vascular and amyloid burden, such

mild vascular burden in our subjects would be rather favorable to achieve the goal.

There were no significant correlation of folate and vitamin B_{12} with brain atrophy. Some previous study reported plasma folate level was associated with brain atrophy.[39, 40] Small sample size and normal folate level probably weaknen those assocications in our study. In terms of vitamin $B_{12,}$ plasma vitamin B_{12} level itself was known to have low sensitivity and not to reflect actual metabolic deficiency.[41] So, more sensitive marker, like methylmalonic acid or holotranscobalamin[41, 42] should be included as measurement in further study.

Our findings have important therapeutic implications. First, it could be inferred that homocysteine lowering agents should have protective effect on hippocampal atrophy. However, previous randomized controlled trials for supplementation with vitamin B_{12} or folate yielded conflicting results,[43, 44] and RCT in patients with mild to moderate AD showed no benefits of using B vitamin on slowing cognitive decline.[45] Possible explanation for those results is that neurotoxic effects, in the case of severe AD, might be over-ridden by Aβ pathology. Eventually, single therapy with B vitamin was not enough to protect brain atrophy or cognitive decline in AD. In subjects with mild Aβ burden like MCI group, Hcy lowering agents slowed rate of brain atrophy.[10] Second, neurotoxic effect of Hcy on hippocampus was independent of Aβ and vascular pathology. So, it could be expected that Hcy lowering supplementations would bring additive benefits when combined with drugs targeting amyloid or vascular pathology. In line with this hypothesis, one RCT showed folic acid acted synergistically with cholinesterase inhibitor to improve functional activity in AD patients.[46]

There are a couple of limitations in our study. First, due to cross-sectional design, we should be cautious to make an interpretation for the relationship between plasma tHcy level and hippocampal atrophy as causative effect of homocysteine on the hippocampus. Further longitudinal studies are needed to demonstrate the causality. Second, we found the direct association of plasma tHcy only with hippocampus, but not with whole brain volume. As previously mentioned, relatively small sample size might make it difficult to find the independent relationship of homocysteine with WBV. The regional specificity of homocysteine effect on hippocampus was not fully clarified yet, and further studies for larger study population are needed.

In conclusion, our finding of the independent negative association between plasma tHcy and hippocampal volume suggests that homocysteine has a direct adverse effect, not mediated by cerebral Aβ deposition and vascular burden, on the hippocampus even within normal range of plasma level. The result further implicates that potential homocysteine lowering approaches could be helpful to reduce hippocampal damage, even in cases unrelated to AD or cerebrovascular disease process. Such homocysteine lowering approaches may have an additive neuroprotective effect when combined with anti-amyloid therapeutics in AD or vascular risk-lowering drugs in cerebrovascular diseases.

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국문 초록

목적: 정상노인, 경도인지장애, 알츠하이머병에서 뇌 베타아밀로이드 축적과 혈관 위험 요인에 의한 영향을 배제한 뒤 혈장 호모시스테인이 전체 뇌 용적과 해마 용적에 미치는 직접적인 영향을 살펴보고자 하였다.

방법: 인지적 정상 노인 14명, 경도인지장애 19명, 알츠하이머병 24명을 대상으로 연구를 진행했다. 모든 참가자는 뇌 자기공명영상과 피츠버그 컴파운드 비를 이용한 양전자 단층 촬영 검사를 시행하였으며 당뇨, 고혈압, 고지혈증, 뇌졸중, 일과성 뇌허혈증의 과거력 유무를 포함한 혈관적 위험 정도를 평가하였다. 호모시스테인, 비타민 B_{12}, 엽산의 혈장 농도도 함께 측정하였다.

결과: 다중선형 회기분석을 시행한 결과 나이, 성별, 교육수준, 아포지단백 e4 유무뿐만 아니라 뇌 베타아밀로이드 축적이나 혈관성 위험의 영향을 배제했을 때에도 호모시스테인이 해마의 위축에 유의미한 영향을 주는 것으로 나왔다. 반면에, 호모시스테인의 혈장 농도와 전체 뇌 용적 사이에는 유의미한 상관관계가 없었다.

결론: 본 연구에서 보여진 혈장 호모시스테인 농도와 해마 용적 간의 음의 상관 관계는 뇌 베타아밀로이드 축적이나 혈관성 위험을 통한 작용과는 독립적으로 호모시스테인이 해마에 직접적인 신경독성 효과를 가진다는 것을 시사한다.

주요어: 호모시스테인, 해마, 베타 아밀로이드 축적, 혈관성 위험, 알츠하이머병, 경도인지장애

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