

# Association of Carotid and Intracranial Stenosis with Alzheimer's Disease Biomarkers

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## Research

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# Abstract

**Background** To clarify whether atherosclerosis of the carotid and intracranial arteries is related to Alzheimer's disease (AD) pathology in vivo, we investigated the associations of carotid and intracranial artery stenosis with cerebral beta-amyloid (A $\beta$ ) deposition and neurodegeneration in middle- and old-aged individuals. Given the differential progression of A $\beta$  deposition and neurodegeneration across clinical stages of AD, we focused separately on cognitively normal (CN) and cognitively impaired (CI) groups.

**Methods** A total of 281 CN and 199 CI (mild cognitive impairment and AD dementia) subjects underwent comprehensive clinical assessment, [11C] Pittsburgh Compound B positron emission tomography, and magnetic resonance (MR) imaging including MR angiography. We evaluated extracranial carotid and intracranial arteries for the overall presence, severity (i.e. number and degree of narrowing) and location of stenosis.

**Results** We found no associations between carotid and intracranial artery stenosis and cerebral A $\beta$  burden in either CN or CI group. In terms of AD-related neurodegeneration, exploratory univariate analyses showed associations between the presence and severity of stenosis and neurodegeneration biomarkers of AD (i.e. reduced hippocampal volume [HV] and cortical thickness in the AD-signature regions) in both CN and CI groups. In confirmatory multivariate analyses controlling for demographic covariates and diagnosis, the association between number of stenotic intracranial arteries  $\geq 2$  and reduced HV in the CI group remained significant.

**Conclusions** Neither carotid nor intracranial artery stenosis appears to be associated with brain A $\beta$  burden, while intracranial artery stenosis is related to amyloid-independent neurodegeneration, particularly hippocampal atrophy. These observations support the importance of proper management of intracranial artery stenosis for delaying the progression of AD neurodegeneration and related cognitive decline.

## Background

Previous studies indicate the association between atherosclerosis of the carotid and intracranial arteries and Alzheimer's disease (AD)-related cognitive impairment (CI), including dementia and mild cognitive impairment (MCI) [1–4]. However, the underlying mechanisms for the link remain unclear. Most previous studies dealing with this issue were based on postmortem brain analyses and the findings were controversial [5–13]. A couple of small scale studies on patients with severe cerebral hypoperfusion yielded inconsistent findings on the relationship between very severe atherosclerosis and amyloid deposition [14, 15]. Although a recent study using high-resolution vessel wall magnetic resonance (MR) imaging reported that intracranial atherosclerotic plaque or stenosis were not associated with beta-amyloid (A $\beta$ ) deposition in nondemented adults [16], knowledge of the association between carotid and intracranial atherosclerosis and variable AD pathologies, neurodegeneration as well as A $\beta$  deposition in the living human brain remains limited.

The relationships between carotid and intracranial artery atherosclerosis and in vivo AD pathologies may be complicated because A $\beta$  deposition begins at the preclinical or cognitively normal (CN) stage of AD and almost saturates in the CI stages of AD, while regional neurodegeneration gradually progresses from MCI to dementia stage [17]. Given the differential progression of A $\beta$  deposition and neurodegeneration across the clinical stages of AD, therefore, a clinical stage-specific approach, separately focusing on the CN stage and CI stage could be helpful.

We aimed to investigate the associations between carotid and intracranial artery stenosis systematically measured on MR angiography and AD biomarkers including cerebral A $\beta$  deposition and regional neurodegeneration in a large number of older adults including both CN and CI groups.

## Methods

### Participants

This study is part of the Korean Brain Aging Study for Early Diagnosis and Prediction of Alzheimer's Disease (KBASE), an on-going prospective, community-based cohort study [18]. As of February 2017, a total of 480 older adults consisting of 281 CN and 199 CI (mild cognitive impairment and AD dementia) subjects were initially recruited. The inclusion criteria for the CN group were (a) aged 55–90 years, (b) no diagnosis of MCI or dementia and (c) Clinical Dementia Rating (CDR) score of 0. For the MCI group, individuals 55-90 years old who fulfilled the core clinical criteria for diagnosis of MCI according to the recommendations of the National Institute on Aging- Alzheimer's Association (NIA-AA) guidelines [19] were included as follows: (a) memory complaints corroborated by the patient, an informant, or clinician, (b) objective memory impairment for age, education, and gender (*i.e.*, at least 1.0 SD below the respective age, education, and gender-specific mean for at least one of the four episodic memory tests included in the Korean version of Consortium to Establish a Registry for Alzheimer's Disease (CERAD-K) neuropsychological battery [Word List Memory, Word List Recall, Word List Recognition and Constructional Recall test]); (c) largely intact functional activities; and (d) no dementia. The global CDR score of all MCI individuals was 0.5. For the AD dementia group, participants 55-90 years old who fulfilled the following inclusion criteria were recruited: (a) criteria for dementia in accordance with the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV-TR), (b) the criteria for probable AD dementia in accordance with the NIA-AA guidelines [20], and (c) a global CDR score of 0.5 or 1. For all groups, individuals with the following conditions were excluded from the study: 1) presence of major psychiatric illness; 2) significant neurological or medical condition or comorbidities that could affect mental function; 3) contraindications to MRI (*e.g.*, pacemaker, claustrophobia); 4) illiteracy; 5) presence of significant visual/hearing difficulty; severe communication or behavioral problems that would make clinical examination or brain scan difficult; 6) taking an investigational drug; and, 7) pregnant or breastfeeding. More detailed information on recruitment of the KBASE cohort was described in our previous report [18].

### Standard protocol approval, registration, and patient consent

This study protocol was approved by the Institutional Review Boards of Seoul National University Hospital and SNU-SMG Boramae Medical Center, Seoul, South Korea. The participants and/or their legal representatives provided written informed consent.

## **Clinical assessment**

All participants were administered comprehensive clinical and neuropsychological assessments by trained psychiatrists and neuropsychologists based on the KBASE assessment protocol which incorporates the CERAD-K [18]. Blood samples were collected to determine apolipoprotein E  $\epsilon$ 4 allele (APOE4) carrier status. Vascular risk factors, including hypertension, diabetes mellitus, hyperlipidemia, coronary artery disease, transient ischemic attack and stroke were evaluated via systematic interview by trained nurses, and vascular risk score (VRS) was calculated for the number of vascular risk factors present and reported as a percentage [18].

## **Image acquisition, preprocessing and measurement of vessel stenosis and AD biomarkers**

All subjects underwent simultaneous three-dimensional (3D) [ $^{11}\text{C}$ ] Pittsburgh compound B (PiB)-positron emission tomography (PET) and 3D T1-weighted MRI using the 3.0T Biograph mMR (PET-MR) scanner (Siemens, Washington DC, USA). The vascular protocol including 3D time-of-flight (TOF)-MR angiography was also administered by trained MRI technologists. Acquisition parameters for 3D TOF-MR angiography, 3D T1-weighted images, fluid attenuated inversion images are described in elsewhere (See Additional file 1).

### ***Systematic evaluation of stenosis on MR angiography***

Diagnosis of extracranial carotid and intracranial arterial stenosis was reached by the consensus between two qualified neuroradiologists (KMK and CHS) blinded to the participants' clinical information. We recorded the overall presence, number and the degree of detectable stenotic lesions in the following 13 arterial segments: right and left proximal cervical internal carotid artery (pICA), right and left intracranial ICA, right and left anterior, middle, and posterior cerebral arteries, right and left intracranial vertebral artery, and basilar artery. For the extracranial carotid artery, the degree of stenosis was measured according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [21] using maximum-intensity projections and source images of the bifurcation of the carotid artery. In cases of intracranial arterial stenosis, the degree of stenosis was calculated based on maximum-intensity projections and source images using the method published for the Warfarin-Aspirin Symptomatic Intracranial Disease Study [22]: percent stenosis =  $[(1 - (D_{\text{stenosis}}/D_{\text{normal}}))] \times 100$ . In the case of an artery with multiple stenotic lesions, the most severe degree was selected. Based on the above quantitative data, participants were categorized into stenosis-positive (stenosis+) vs stenosis-negative (stenosis-) groups according to the stenosis measurements for extracranial carotid and intracranial arteries as follows: i) overall presence of any detectable stenosis; ii) severity (*i.e.*, the degree of stenosis  $\geq 50\%$ , and number of stenotic arteries  $\geq 2$ ). In terms of the location of intracranial arterial stenosis, the presence of detectable stenosis in the anterior circulation and posterior circulations were also evaluated. As there

were only very limited numbers of cases with  $\geq 50\%$  stenotic lesions in the extracranial carotid arteries (1 of 281 subjects in the CN group and 1 of 196 subjects in the CI group), and those with bilateral extracranial carotid stenosis (6 of 281 subjects in the CN group and 6 of 196 subjects in the CI group) in our sample, these measurements could not be applied to the extracranial carotid arteries and only available for evaluation of intracranial arterial stenosis. Interobserver agreement for stenosis was determined by calculating the Cohen's kappa correlation coefficient from 125 randomly selected individuals. The kappa values were 0.715 for extracranial carotid stenosis, 0.869 for any intracranial stenosis, 0.715 for number of stenotic intracranial arteries  $\geq 2$ , 0.802 for anterior circulation stenosis, and 1 for posterior circulation stenosis. The kappa value for  $\geq 50\%$  stenosis was 0.301 due to the very low prevalence of  $\geq 50\%$  stenosis despite the high degree of interobserver agreement (5 of 125 [4%] vs. 7 of 125 [5.6%]).

### ***Beta-amyloid (A $\beta$ ) biomarker of AD***

For measurement of A $\beta$  biomarker of AD, a 30-minute emission scan was obtained 40 minutes after injection of intravenous administration of 555 MBq of [ $^{11}\text{C}$ ]PiB (range, 450-610 MBq). The PiB-PET data collected in list mode were processed for routine corrections such as uniformity, UTE-based attenuation, and decay corrections, and were reconstructed into a  $344 \times 344$  image matrix using iterative methods (5 iterations with 21 subsets). The image pre-processing steps were performed using Statistical Parametric Mapping 8 (SPM8; <http://www.fil.ion.ucl.ac.uk/spm>) implemented in Matlab 2014a (MathWorks, Natick, MA, USA). Static PiB-PET images were co-registered to individual T1 structural images and transformation parameters for spatial normalization of individual T1 images to a standard Montreal Neurological Institute (MNI) template were calculated. The inverse transformation of parameters to transform coordinates from the automatic anatomic labelling (AAL) 116 atlas [23] into an individual space for each subject (resampling voxel size =  $1 \times 0.98 \times 0.98$  mm) was performed using IBASPM (Individual Brain Atlases using Statistical Parametric Mapping) software in MATLAB. To extract gray matter (GM) and exclude the non-GM portions of the atlas (*i.e.*, white matter [WM] and cerebrospinal fluid space), a GM mask was applied for each individual. The mean regional  $^{11}\text{C}$ -PiB uptake values from cerebral regions were extracted using the individual AAL116 atlas from T1-coregistered PiB-PET images. Cerebellar GM was used as the reference region for quantitative normalization of cerebral PiB uptake values, due to its relatively low A $\beta$  deposition [24], with a probabilistic cerebellar atlas (Institute of Cognitive Neuroscience, UCL; Cognitive Neuroscience Laboratory, Royal Holloway, University of London, UK) which was transformed into individual space as described above. The AAL algorithm and a region combining method [25] were applied to determine regions of interest (ROIs) to characterize the  $^{11}\text{C}$ -PiB retention levels in the frontal, lateral parietal, posterior cingulate-precuneus, and lateral temporal regions. A global A $\beta$  retention value (standardized uptake value ratio, SUVR) was generated by dividing the voxel-weighted mean value of the four ROIs by the mean cerebellar uptake value [25-27].

### ***Neurodegeneration biomarkers of AD***

All T1-weighted MR images were automatically segmented using FreeSurfer version 5.3 (<http://surfer.nmr.mgh.harvard.edu/>) with manual correction of minor segmentation errors. As neurodegeneration biomarkers of AD, both AD-signature cortical thickness (ADT; *i.e.*, mean cortical thickness obtained from AD-signature regions) and hippocampal volume adjusted for intracranial volume (HVa) were measured used as described previously [26]. First, ADT was defined as the mean cortical thickness values of AD-signature regions including the entorhinal, inferior temporal, middle temporal, and fusiform gyrus, based on the Desikan–Killany atlas [28]. Second, to obtain HVa, left and right hippocampi were first extracted and added together to yield the total hippocampal volume (HV). Then, the volume deviating from the expected total HV according to intracranial volume (ICV) in the reference group (*i.e.*, young cognitively normal group of the study cohort) was calculated to obtain HVa [29]. Detailed information on the characteristics of the reference group for HVa were reported previously [29].

### ***White matter hyperintensities***

The volume of white matter hyperintensities (WMH) on fluid attenuated inversion images was calculated using a validated automatic procedure [30] with two modifications, as follows: first, an optimal threshold of 70 instead of 65 in the original reference was applied because it was more suitable for our data; second, diffusion-weighted imaging was not used in the present automated procedure as there were no participants with acute cerebral infarcts in our study population. WMH candidate images were used to extract WMH volumes based on lobar ROIs in the native space for each subject [31].

### **Statistical Analysis**

All statistical analyses were performed focusing on CN and CI separately. To investigate whether a measure of extracranial carotid or intracranial stenosis was associated with AD biomarkers (*i.e.*, global A $\beta$  deposition, ADT, and HVa) within the CN or CI group, two steps of analysis including exploratory and confirmative steps were conducted. Exploratory univariate analyses were performed with independent t test to compare the quantitative values of AD biomarkers between stenosis+ and stenosis– groups. The AD biomarkers with  $p < 0.05$  in exploratory univariate analyses were selected for the next confirmatory multivariate analyses. Confirmative multivariate analyses were conducted for the selected biomarker with analysis of covariance (ANCOVA) adjusting for age, sex and APOE4 carrier status for the CN group, and age, sex APOE4 carrier status and diagnosis (MCI or AD dementia) for the CI group. The Bonferroni correction method was applied to multiple comparisons using  $p < 0.05/\text{No. of confirmatory analyses}$  within each cognitive group. ANCOVA models with WMH volume as an additional covariate were also tested to evaluate the mediating effect of WM lesions. All statistical analyses were performed using IBM SPSS Statistics 23 (SPSS Inc., Chicago, IL, USA), and  $p < 0.05$  (two-sided) was taken to indicate statistical significance unless otherwise specified.

### **Data Availability**

The datasets generated and analyzed during the present study are not publicly available, owing to ethics considerations and privacy restriction. Data may be available from the corresponding author once

approval from the Institutional Review Board of the Seoul National University Hospital, South Korea has been sought.

## Results

### Characteristics of the participants

Data on the characteristics of the participants are presented in Table 1. The CI group consisted of 129 subjects with MCI and 70 subjects with AD dementia. Three cases were excluded from the evaluation of extracranial carotid stenosis due to motion artefacts.

### Association of carotid and intracranial stenosis with AD biomarkers in the CN group

In the exploratory step of the analyses, we found no significant differences in global A $\beta$  deposition between CN subjects with vs. those without any type of stenosis (Table 2). In contrast, with regard to neurodegeneration biomarkers, ADT was significantly reduced in CN subjects in the stenosis+ group compared with CN subjects in the stenosis- group for the presence of any extracranial carotid stenosis, presence of any intracranial stenosis, number of stenotic intracranial arteries  $\geq 2$ , anterior circulation stenosis and posterior circulation stenosis (all  $p < 0.05$ ; Table 2). In addition, HVa was significantly reduced in the stenosis+ CN group compared to the stenosis- CN group for the presence of any intracranial arterial stenosis. Next, for confirmatory analyses in the CN group, we further investigated the associations between ADT and each of the abovementioned five measurements of stenosis that showed association in exploratory univariate analyses ( $p < 0.05$ ), as well as the association between HVa and presence of any intracranial stenosis, after controlling for the effects of age, sex and APOE4 carrier status. When Bonferroni corrected p-value ( $p < 0.05/6 = 0.008$ ) was applied, these associations were not significant in the confirmatory analyses (ADT,  $p = 0.121$  for any extracranial carotid stenosis,  $p = 0.104$  for any intracranial stenosis,  $p = 0.113$  for number of stenotic intracranial arteries  $\geq 2$ ,  $p = 0.254$  for anterior circulation stenosis and  $p = 0.030$  for posterior circulation stenosis, respectively; HVa,  $p = 0.748$  for any intracranial stenosis), although posterior circulation stenosis showed a trend for association with reduced ADT ( $p = 0.030$ ).

### Association of carotid and intracranial stenosis with AD biomarkers in the CI group

In the CI group, exploratory univariate analyses found an association between anterior circulation stenosis and lower global A $\beta$  deposition ( $p = 0.049$ , Table 3). However, it was not significant in the confirmatory step when controlling for age, gender, APOE4 carrier status and clinical diagnosis (MCI vs. AD dementia) ( $p = 0.183$ ). In terms of exploratory univariate analyses of neurodegeneration biomarkers, there were no differences in ADT between stenosis+ and stenosis- groups for any type of stenosis in CI subjects (Table 3). However, the presence of any intracranial arterial stenosis and number of stenotic intracranial arteries  $\geq 2$  were associated with reduced HVa in CI subjects ( $p = 0.047$  and  $p = 0.008$ , respectively; Table 3). These two associations were selected for subsequent confirmatory multivariate analyses after controlling for age, gender, APOE4 carrier status and clinical diagnosis. Bonferroni

corrected  $p < 0.05/2 = 0.025$  was used as a statistical threshold. As a result, CI subjects with number of stenotic intracranial arteries  $\geq 2$  had significantly lower HVa than those without when controlling for age, gender, APOE4 carrier status and clinical diagnosis ( $p = 0.021$ ; Table 3 and Figures 1, 2). The results did not change even after additional adjustment for WMH volume ( $p = 0.014$ ). On the other hand, the association between presence of any intracranial stenosis and lower HVa in the CI group did not remain significant in confirmatory analysis ( $p = 0.597$ ).

## Discussion

This study was performed to investigate the associations of both extracranial carotid and intracranial artery stenosis with in vivo AD pathologies, i.e., global A $\beta$  burden and neurodegeneration in a large number of older adults, focusing on CN and CI groups separately. Global A $\beta$  burden was not related to any vessel stenosis in either group. With regard to neurodegeneration, the CN group did not show any significant associations between carotid and intracranial artery stenosis and ADT or HVa. In contrast, in the CI group, number of stenotic intracranial arteries  $\geq 2$  was significantly associated with lower HVa even after adjusting for age, gender, APOE4 carrier status and diagnosis (i.e., MCI vs. AD dementia).

A number of previous postmortem brain studies investigated the association between cerebral atherosclerosis and A $\beta$  burden, but the results were controversial [5–11]. While a number of previous autopsy studies reported a significant association between intracranial atherosclerosis and neuritic plaques in AD [5–7, 11], several others found no such associations [8–10]. Recently, a community based study in adults without dementia reported that intracranial atherosclerotic plaque or stenosis was not associated with A $\beta$  deposition in the brain [16]. Our results in the CN and CI groups are in agreement with the study [16] in regards to the relationship between carotid and intracranial stenosis and global A $\beta$  burden. As both cerebral atherosclerosis and AD are common in the elderly, the possibility of coincidence cannot be excluded in postmortem studies with positive association between the two.

In contrast to global A $\beta$  burden, intracranial artery stenosis was associated with neurodegeneration biomarkers in the CI group. In particular, number of stenotic intracranial arteries  $\geq 2$  was significantly associated with lower hippocampal volume in CI subjects in confirmatory multivariate analysis. Some previous studies identified intracranial atherosclerosis as an independent risk factor for cerebral atrophy [12, 13]. In these studies, however, intracranial atherosclerosis was assessed based only on cavernous ICA calcification on computed tomography [13] or pathological examination of the circle of Willis [32]. In contrast, we evaluated stenosis of all cerebral arteries. With this approach and strict control for multiple testing errors, we confirmed the association between intracranial artery stenosis and HVa in the CI group. The association remained significant even after controlling for WMH volume, suggesting that intracranial artery stenosis affects hippocampal atrophy independently of changes in the WM. Hippocampal atrophy is a validated neurodegeneration biomarker of AD and is closely correlated with early cognitive decline in AD [33]. Therefore, the association of intracranial stenosis with decreased HVa, together with no association with A $\beta$  burden, in the CI group indicates that intracranial stenosis contributes to the development of AD-related CI via A $\beta$ -independent neurodegeneration of the hippocampus.

In the CN group, while exploratory univariate analyses showed relations between various types of stenosis and ADT or HVa in CN, such relations were not confirmed by multivariate analyses with a strict statistical threshold. Nevertheless, ADT tended to be lower in subjects with posterior circulation stenosis than those without ( $p = 0.03$ ), although the difference was not statistically significant after Bonferroni correction. In a previous postmortem study which reported the association between intracranial artery atherosclerosis and AD dementia, the posterior cerebral artery (PCA), one of the posterior circulation, showed the most severe atherosclerosis among the circle of Willis arteries in AD dementia patients [7]. Therefore, the potential association between posterior circulation stenosis and ADT deserves attention. Actually, the AD-signature regions, where we measured cortical thickness, include the entorhinal, inferior temporal, and fusiform gyri that receive blood supply from the PCA.

We found no significant associations between extracranial carotid stenosis and AD biomarkers regardless of the presence of cognitive impairment. Some previous in vivo studies with very small sample sizes investigated the associations between severe carotid stenosis or occlusion and A $\beta$  deposition and yielded contradictory findings. While one study using [ $^{18}\text{F}$ ] AV-45 PET indicated that A $\beta$  deposition increased in dementia patients with unilateral carotid artery stenosis, and its distribution was lateralized to the side of stenosis [14], a more recent study using [ $^{18}\text{F}$ ] flutemetamol PET indicated that cerebral hypoperfusion caused by unilateral occlusion of the ICA did not induce brain accumulation of A $\beta$  [15]. In terms of neurodegeneration, our results were inconsistent with several previous studies that showed increased carotid intima–media thickness and carotid stenosis were associated with decreased total brain volume [3, 34]. This discrepancy may have been due to the severity of stenosis. Only a very small number of subjects with  $\geq 50\%$  stenosis in the extracranial carotid artery were included in the present study (1 of 281 subjects in the CN group and 1 of 196 subjects in the CI group), while many previous studies indicated associations of the degree of severity [3, 34] or severe carotid atherosclerosis [1, 35] with brain atrophy.

To our knowledge, this is the first study to investigate the association between MR angiography-measured vessel stenosis in the extracranial carotid and intracranial arteries and in vivo AD pathologies, including both A $\beta$  deposition and neurodegeneration, in a large sample of older adults, focusing separately on CN and CI groups. However, there were some limitations in our study. First, due to its cross-sectional design, we cannot make conclusions regarding the cause and effect relationship between carotid and intracranial artery stenosis and in vivo AD pathologies. Second, although we included a relatively large number of subjects, the frequencies of stenosis, particularly extracranial carotid stenosis, number of stenotic intracranial arteries  $\geq 2$ , and posterior circulation stenosis, were relatively low in both CN and CI groups, which may have reduced statistical power and made it difficult to identify significant associations after multiple comparison correction. Carotid and intracranial artery stenosis has not been a common finding in community-dwelling subjects [36, 37], with reported prevalence rates of asymptomatic intracranial stenosis ranging from 5.9–24.5% [37]. In addition, the prevalence of cervical carotid artery stenosis varies significantly with ethnicity, and it was reported to be particularly uncommon in a Korean

population-based screening cohort [36]. Therefore, our study population seemed to reflect the prevalence of asymptomatic carotid and intracranial stenosis in the general Asian population.

## Conclusions

In conclusion, our findings suggested that neither carotid nor intracranial artery stenosis are associated with brain A $\beta$  burden, while intracranial artery stenosis is related to amyloid-independent neurodegeneration, particularly hippocampal atrophy. This supports the importance of proper management of intracranial artery stenosis for delaying the progression of AD neurodegeneration and related cognitive decline.

## Abbreviations

3D: three-dimensional; AAL: automatic anatomic labelling; A $\beta$ : beta-amyloid; AD: Alzheimer's disease; ADT: AD-signature cortical thickness; ANCOVA: analysis of covariance ; APOE4: apolipoprotein E  $\epsilon$ 4 allele; CDR: Clinical Dementia Rating; CERAD-K: Consortium to Establish a Registry for Alzheimer's Disease; CI: cognitive impairment; CN: cognitively normal; DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders 4th Edition ; GM: gray matter; HV: hippocampal volume ; HVa: hippocampal volume adjusted for intracranial volume; IBMSPM: Individual Brain Atlases using Statistical Parametric Mapping; ICA: internal carotid artery; ICV: intracranial volume ; KBASE: Korean Brain Aging Study for Early Diagnosis and Prediction of Alzheimer's Disease; MCI: mild cognitive impairment; MNI: Montreal Neurological Institute; MR: magnetic resonance; NASCET: North American Symptomatic Carotid Endarterectomy Trial; NIA-AA: National Institute on Aging- Alzheimer's Association; PET: positron emission tomography; PiB: Pittsburgh compound B; ROIs: regions of interest; SPM: statistical Parametric Mapping; SUVR: standardized uptake value ratio; TOF: time-of-flight; VRS: vascular risk score; WM: white matter; WMH: white matter hyperintensities

## Declarations

### Ethics approval and consent to participate

This study protocol was approved by the Institutional Review Boards of Seoul National University Hospital and Seoul National University-Seoul Metropolitan Government Boramae Center, and all subjects provided written informed consent.

### Consent for publication

Not applicable

### Availability of data and material

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

### **Competing interests**

Not applicable

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### **Authors' contributions**

KMK, MSB, CHS, and DYL contributed to the conception and design of the study. KMK, MSB, CHS, and DYL contributed to drafting the text and preparing figures. All authors contributed to the acquisition and analysis of data. JHL, DY, HJC, EL, YL, JYL, YKK, BKS contributed the acquisition, analysis and interpretation of data.

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## Tables

Table 1. Characteristics of participants

Variables	CN (N=281)	CI (N=199)
<i>Demographic and clinical characteristics</i>		
Age, y	69.1 ± 8.1	72.9 ± 7.4
Females	146 (52.0%)	134 (67.3%)
Education, y	12.3 ± 4.4	9.7 ± 4.9
APOE4 carriers	52 (18.5%)	83 (41.7%)
Global CDR (0/0.5/1)	281/0/0	0/153/46
CDR-SOB	0.00 ± 0.06	2.71 ± 1.96
Hypertension	133 (47.3%)	99 (49.7%)
Diabetes Mellitus	46 (16.4%)	34 (17.1%)
Coronary artery disease	16 (5.7%)	19 (9.5%)
Hyperlipidemia	123 (43.8%)	80 (40.2%)
Stroke	0 (0.0%)	0 (0.0%)
TIA	2 (0.7%)	1 (0.5%)
VRS	17.4 ± 16.1	18.0 ± 16.8
WMH volume (cm <sup>3</sup> ) <sup>a</sup>	5.68 ± 5.32	6.35 ± 5.19
<i>AD biomarkers</i>		
Global Aβ deposition (SUVR)	1.184 ± 0.239	1.621 ± 0.156
<i>Neurodegeneration biomarkers</i>		
ADT (mm)	2.866 ± 0.174	2.584 ± 0.286
HVa (mm <sup>3</sup> )	-759 ± 838	-2132 ± 1219
<i>Large vessel stenosis on MR angiography</i>		
Overall presence of detectable stenosis		
Any extracranial carotid stenosis	23 (8.2%)	22 <sup>b</sup> (11.2%)
Any intracranial stenosis	77 (27.4%)	71 (35.7%)
<i>Severity</i>		
≥ 50 % intracranial stenosis	19 (6.8%)	21 (10.6%)
Number of stenotic intracranial arteries ≥ 2	44 (15.7%)	38 (19.1%)
<i>Location</i>		

Anterior circulation stenosis	69 (24.6%)	61 (30.7%)
Posterior circulation stenosis	21 (7.5%)	21 (10.6%)

Note. Data are presented as mean  $\pm$  SD or n (%).

<sup>a</sup> Data for 422 individuals were available (256 CN and 166 CI)

<sup>b</sup> Data for 196 CI individuals were available.

CN, cognitively normal; CI, cognitively impaired : CDR-SOB, Clinical Dementia Rating sum of box; TIA, transient ischemic attack; VRS, vascular risk factor score; WMH, white matter hyperintensity; ADT, Alzheimer’s disease signature cortical thickness; HVa, Hippocampal volume adjusted for intracranial volume

**Table 2. Exploratory univariate analyses for the association between extracranial carotid and intracranial arterial stenosis and AD biomarkers in the CN group.**

AD biomarker	Variables			Overall presence of detectable stenosis					
				Any extracranial carotid stenosis			Any intracranial stenosis		
				Stenosis-	Stenosis+	P-value	Stenosis-	Stenosis+	P-value
			N	258	23		204	77	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)			1.185 $\pm$ 0.245	1.173 $\pm$ 0.160	0.810	1.182 $\pm$ 0.243	1.190 $\pm$ 0.230	0.782
Neurodegeneration biomarker	ADT (mm)			2.877 $\pm$ 0.170	2.743 $\pm$ 0.173	< 0.001*	2.890 $\pm$ 0.171	2.804 $\pm$ 0.165	< 0.001*
	HVa (mm <sup>3</sup> )			-732 $\pm$ 810	-1067 $\pm$ 1076	0.066	-686 $\pm$ 832	-953 $\pm$ 827	0.017*
<b>Severity</b>									
				$\geq$ 50 % intracranial stenosis			Number of stenotic intracranial arteries $\geq$ 2		
				Stenosis-	Stenosis+	P-value	Stenosis-	Stenosis+	P-value
			N	262	19		237	44	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)			1.179 $\pm$ 0.234	1.258 $\pm$ 0.293	0.165	1.189 $\pm$ 0.242	1.157 $\pm$ 0.221	0.409
Neurodegeneration biomarker	ADT (mm)			2.871 $\pm$ 0.175	2.801 $\pm$ 0.136	0.091	2.882 $\pm$ 0.169	2.780 $\pm$ 0.178	< 0.001*
	HVa (mm <sup>3</sup> )			-746 $\pm$ 838	-937 $\pm$ 837	0.338	-726 $\pm$ 840	-938 $\pm$ 810	0.123
<b>Location</b>									
				Anterior circulation			Posterior circulation		
				Stenosis-	Stenosis+	P-value	Stenosis-	Stenosis+	P-value
			N	212	69		260	21	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)			1.187 $\pm$ 0.245	1.175 $\pm$ 0.220	0.711	1.185 $\pm$ 0.241	1.175 $\pm$ 0.212	0.854
Neurodegeneration biomarker	ADT (mm)			2.884 $\pm$ 0.172	2.811 $\pm$ 0.167	0.002*	2.876 $\pm$ 0.170	2.744 $\pm$ 0.181	0.001*
	HVa (mm <sup>3</sup> )			-710 $\pm$ 837	-911 $\pm$ 828	0.082	-760 $\pm$ 836	-746 $\pm$ 875	0.943

Note. Data for continuous variables presented as means  $\pm$  SD.

CN, cognitively normal; SUVR, standardized uptake value ratio; ADT, Alzheimer's disease signature cortical thickness; HVa, Hippocampal volume adjusted for intracranial volume

\* $p < 0.05$

Table 3. Exploratory univariate analyses for the association between extracranial carotid and intracranial arterial stenosis and AD biomarkers in the CI group.

AD biomarker	Variables		Overall presence of detectable stenosis					
			Any extracranial carotid stenosis			Any intracranial stenosis		
			Stenosis-	Stenosis+	P-value	Stenosis-	Stenosis+	P-value
		N	174	22		128	71	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)		1.627 $\pm$ 0.530	1.609 $\pm$ 0.412	0.857	1.648 $\pm$ 0.539	1.573 $\pm$ 0.472	0.304
Neurodegeneration biomarker	ADT (mm)		2.600 $\pm$ 0.284	2.518 $\pm$ 0.260	0.205	2.596 $\pm$ 0.311	2.561 $\pm$ 0.234	0.376
	HVa (mm <sup>3</sup> )		-2064 $\pm$ 1225	-2396 $\pm$ 947	0.146	-2004 $\pm$ 1262	-2362 $\pm$ 1109	0.047*
<b>Severity</b>								
			$\geq$ 50 % intracranial stenosis			Number of stenotic intracranial arteries $\geq$ 2		
		N	178	21		161	38	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)		1.646 $\pm$ 0.522	1.415 $\pm$ 0.411	0.052	1.640 $\pm$ 0.536	1.544 $\pm$ 0.414	0.234
Neurodegeneration biomarker	ADT (mm)		2.584 $\pm$ 0.290	2.579 $\pm$ 0.254	0.940	2.598 $\pm$ 0.292	2.521 $\pm$ 0.252	0.136
	HVa (mm <sup>3</sup> )		-2087 $\pm$ 1202	-2515 $\pm$ 1327	0.128	-2021 $\pm$ 1227	-2602 $\pm$ 1080	0.008*
<b>Location</b>								
			Anterior circulation			Posterior circulation		
		N	138	61		178	21	
A $\beta$ biomarker	Global A $\beta$ deposition (SUVR)		1.667 $\pm$ 0.535	1.519 $\pm$ 0.456	0.049*	1.623 $\pm$ 0.519	1.605 $\pm$ 0.498	0.878
Neurodegeneration biomarker	ADT (mm)		2.585 $\pm$ 0.309	2.580 $\pm$ 0.224	0.902	2.589 $\pm$ 0.289	2.540 $\pm$ 0.257	0.459
	HVa (mm <sup>3</sup> )		-2047 $\pm$	-2323 $\pm$	0.142	-2100 $\pm$ 1217	-2400 $\pm$ 1236	0.287

Note. Data for continuous variables presented as means  $\pm$  SD.

CI, cognitively impaired; SUVR, standardized uptake value ratio; ADT, Alzheimer's disease signature cortical thickness; HVa, Hippocampal volume adjusted for intracranial volume

\* $p < 0.05$

## Figures

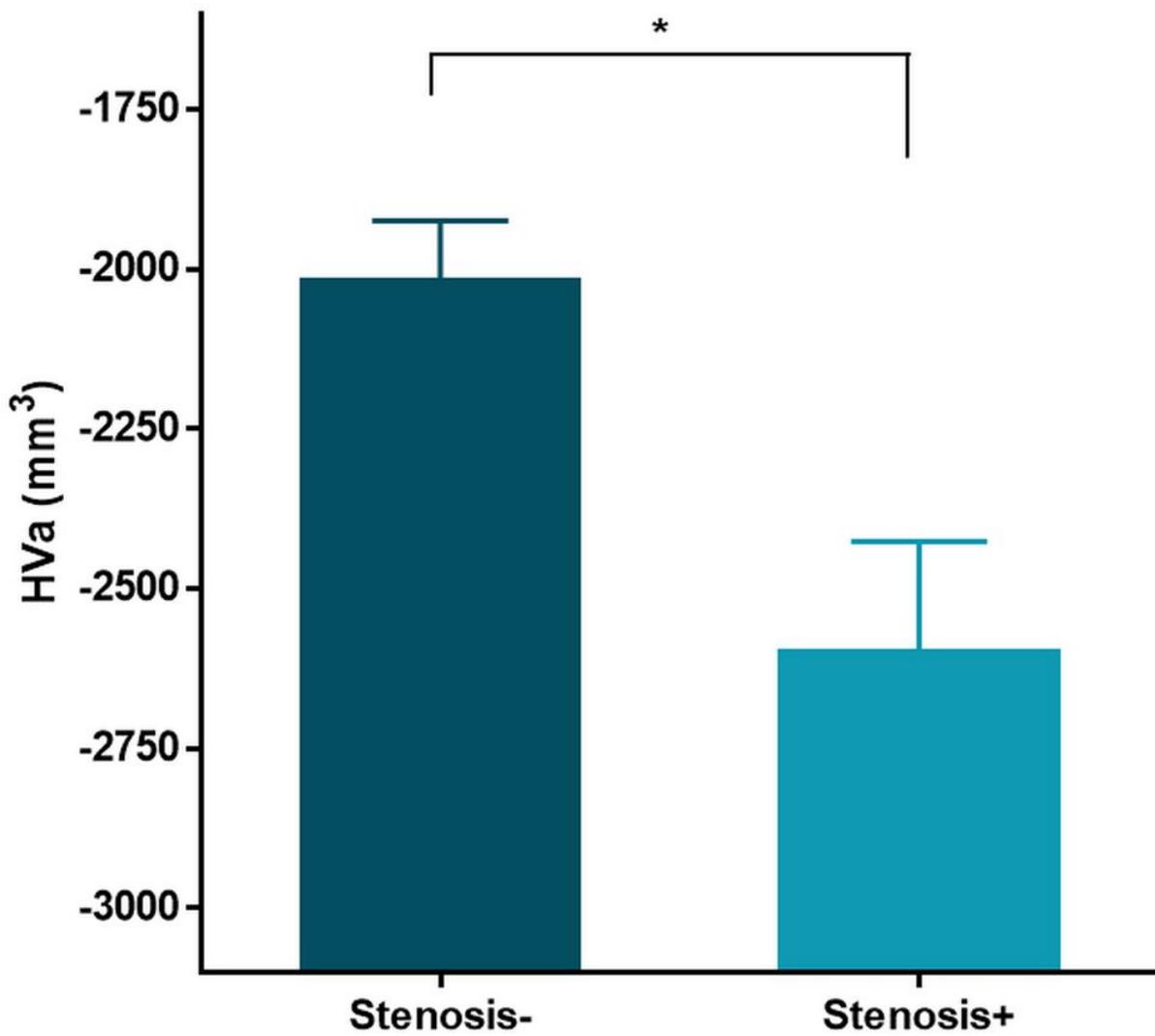


Figure 1

Comparison of HVa between stenosis- and stenosis+ groups for number of stenotic intracranial arteries  $\geq 2$  in CI subjects. \*Adjusted  $p < 0.05$  (after controlling for the effects of age, gender, APOE4 and clinical diagnosis (MCI vs. AD dementia). Bar graph and error bar indicate mean and SEM. HVa, hippocampal volume adjusted for intracranial volume; MCI, mild cognitive impairment; AD, Alzheimer's disease



**Figure 2**

Representative MR angiographic images of intracranial and neck vessels, and coronal sections of T1-weighted images showing medial temporal structures including the bilateral hippocampi of CI individuals in the (A) stenosis- group and (B) stenosis+ group with regard to number of stenotic intracranial arteries  $\geq 2$  (A) stenosis-: MR angiography of 77-year-old woman with MCI with no steno-occlusive lesions in both intracranial and neck vessels. HVa was  $-1052\text{mm}^3$ , and no significant hippocampal atrophy was observed in coronal sections on T1-weighted MRI. (B) Stenosis+: MR angiography of 81-year-old woman with MCI with multifocal intracranial arterial stenosis, while no steno-occlusive lesions were found in the extracranial carotid arteries. HVa was  $-3862\text{mm}^3$ , and coronal sections on T1-weighted MRI indicate bilateral hippocampal atrophy. MR, magnetic resonance; HVa, hippocampal volume adjusted for intracranial volume; MCI, mild cognitive impairment

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