Supplementary Online Content

Lee MJ, Seo SW, Na DL, et al. Synergistic effects of ischemia and β-amyloid burden on cognitive decline in patients with subcortical vascular mild cognitive impairment. *JAMA Psychiatry*. Published online February 19, 2014. doi:10.1001/jamapsychiatry.2013.4506.

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Comparison of demographics between included and excluded patients with svMCI

	Included group (n=67)	Excluded group (n=28)	*P*
Age, y (SD)	73.7 (6.7)	74.5 (9.2)	0.685
Education, y (SD)	9.4 (5.4)	8.4 (5.4)	0.440
Female sex, N (%)	41 (61)	16 (61.5)	0.976
Vascular risk factors			
Hypertension, N (%)	50 (75)	20 (77)	0.818
Diabetes, N (%)	17 (25)	4 (15)	0.301
Hyperlipidemia, N (%)	20 (30)	7 (27)	0.780
Cardiac disease, N (%)	17 (25)	3 (12)	0.145
Stroke, N (%)	13 (19)	5 (19)	0.985

Abbreviations: svMCI = subcortical vascular mild cognitive impairment, MMSE = Mini-Mental Status Exam.

eTable 2A. Spearman's correlation between total PiB retention ratio and MRI markers of ischemia (both log-transformed)

	Total PiB	
	ρ (95% CI)	*P* value
Total WMH	-0.14 (-0.383 - 0.104)	0.261
Total Lacunes	-0.293 (-0.524 - -0.072)	0.017
Total Microbleeds	-0.102 (-0.342 - 0.144)	0.417

eTable 2B. Spearman's correlation between PiB retention ratio and regional WMH volumes, lacunes or microbleeds in frontal region (both log-transformed)

	Frontal PiB	
	ρ (95% CI)	*P* value
Frontal WMH	0.096 (-0.171 - 0.343)	0.447
Frontal Lacunes	-0.25 (-0.482 - 0.004)	0.043
Frontal Microbleeds	-0.095 (-0.321 - 0.127)	0.447

eTable 2C. Spearman's correlation between PiB retention ratio and regional WMH volumes, lacunes or microbleeds in temporal region (both log-transformed)

	Temporal PiB	
	ρ (95% CI)	*P* value
Temporal WMH	0.023 (-0.232 - 0.268)	0.853
Temporal Lacunes	-0.098 (-0.241 - 0.04)	0.435
Temporal Microbleeds	-0.134 (-0.357 - 0.105)	0.283

eTable 2D. Spearman's correlation between PiB retention ratio and regional WMH volumes, lacunes or microbleeds in parietal region (both log-transformed)

	Parietal PiB	
	ρ (95% CI)	*P* value
Parietal WMH	0.006 (-0.245 - 0.261)	0.964
Parietal Lacunes	-0.168 (-0.386 - 0.064)	0.179
Parietal Microbleeds	-0.121 (-0.389 - 0.141)	0.332

eMethods 1

PiB-PET Data acquisition

All subjects underwent a PET scan using a Discovery STe PET/CT scanner (GE Medical Systems, Milwaukee, WI) in a 3-dimensional scanning mode that examined 35 slices of 4.25-mm thickness that spanned the entire brain. The 11C-PiB was injected into an antecubital vein as a bolus with a mean dose of 420 MBq (i.e., range 259–550 MBq). A CT scan was performed for attenuation correction at 60 minutes after the injection. A 30-minute emission static PET scan was then initiated. The specific radioactivity of 11 C-PiB at the time of administration was more than 1,500 Ci/mmol for patients and the radiochemical yield was more than 35%. The radiochemical purity of the tracer was more than 95% in all PET studies.

PiB-PET Data Analysis

PiB PET images were co-registered to individual MRIs, which were normalized to a T1-weighted MRI template. Using these parameters, MRI co-registered PiB PET images were normalized to the MRI template. The quantitative regional values of PiB retention on the spatially normalized PiB images were obtained by an automated volume of interest (VOIs) analysis using the automated anatomical labeling (AAL) atlas. Data processing was performed using SPM Version 5 (SPM5) within Matlab 6.5 (MathWorks, Natick, MA).

To measure PiB retention, we used the cerebral cortical region to cerebellum uptake ratio (UR). The cerebellum was used as a reference region as it did not show group differences. We selected 28 cortical VOIs from left and right hemispheres using the AAL atlas. The cerebral cortical VOIs that were chosen for this study consisted of the bilateral frontal (superior and middle frontal gyri, the medial portion of superior frontal gyrus, the opercular portion of inferior frontal gyrus, the triangular portion of inferior frontal gyrus, supplementary motor area, orbital portion of the superior, middle, and inferior orbital frontal

gyri, rectus and olfactory cortex), posterior cingulate gyri, parietal (superior and inferior parietal, supramarginal and angular gyri, and precuneus), lateral temporal (superior, middle and inferior temporal gyri, and heschl gyri), and occipital (superior, middle, and inferior occipital gyri, cuneus, calcarine fissure, and lingual and fusiform gyri). Regional cerebral cortical URs were calculated by dividing each cortical VOI's UR by the mean uptake of the cerebellar cortex (cerebellum crus1 and crus2). Global PiB uptake ratio was calculated from the volume-weighted average UR of bilateral 28 cerebral cortical VOIs. We defined PiB uptake ratio to be a continuous variable. Patients were considered PiB-positive if their global PiB uptake ratio was more than two standard deviations (PiB retention ratio > 1.5) from the mean of the normal controls.

eMethods 2

MRI acquisition

FLAIR MR images were acquired in the axial plane using the following parameters: axial slice thickness, 2 mm; no gap; repetition time (TR), 11000.0 ms; echo time (TE), 125.0 ms; flip angle, 90°; and matrix size of 512x512 pixels. T2*-weighted gradient-recalled echo MRIs were obtained using the following parameters: axial slice thickness, 5.0 mm; inter-slice thickness, 2 mm; TR, 669 ms; TE 16 ms; flip angle, 18°; and matrix size of 560x 560 pixels.

eMethods 3

Measurement of regional WMH volume

Because the contrasting properties of FLAIR images allow automated segmentation and classification of WMH,[1] we used FLAIR images to quantify WMH. The procedures for measuring regional WMH volume have been previously described.[2] First, we extracted the WMH candidate regions using T1-weighted images to avoid misclassification in the subarachnoid space and CSF interface, which cannot be excluded by intensity threshold or the conventional brain extraction tools. Second, in order to extract WMH, a threshold method was applied to the FLAIR images after using the WMH candidate mask. Even though the threshold value was selected after intensity normalization, segmented results could contain the false positive or negative depending on the extent of WMH. Therefore, if the results were found to contain an error, the threshold value was reselected through visual inspection by two raters.

Extracted WMH were localized and quantified according to the brain lobes (frontal, parietal, temporal, and occipital) by application of prelabeled 3-D probabilistic anatomical atlases using a nonlinear registration-based technique. Eventually, the WMH in the thalamus and basal ganglia were incorporated into the frontal region.

eMethods 4

Assessment of lacunes and microbleeds (MBs) on MRI

Lacunes were defined as small lesions (≤15mm and ≥3mm in diameter) with low signal on T1-weighted images, high signal on T2-weighted images and a perilesional halo on 80 axial slices of FLAIR images. MB were defined as ≤10mm in diameter using criteria proposed by Greenberg *et al.*[28] on 20 axial slices of T2* gradient-recall echo MR images. The numbers of lacunes and MB were counted in four lobar white matter (frontal, parietal,

temporal, and occipital), thalamus, basal ganglia and infratentorial regions. Lacunes or MB in the thalamus and basal ganglia were incorporated into the frontal region because the thalamus and basal ganglia are part of the fronto-subcortical circuit.

eMethods 5

Time intervals between PiB imaging, MRI and neuropsychological tests

There were no differences in time from neuropsychological tests to PiB-PET (median [interquartile range]: 3.0 [1.9 - 5.4] months vs. 2.3 [1.3 - 3.2] months, $P = 0.061$) and time from MRI to PIB-PET (1.6 [0.0 – 4.0] months vs. 2.4 [1.4 - 3.6] months, $P = 0.073$) between aMCI and svMCI subjects. Although differences in time from neuropsychological tests to MRI (0.0 [0.0 - 3.3] months vs. 0.0 [0.0 – 0.0] months, $P = 0.002$) occured, their median differences were negligible.

eMethods 6

Analysis of Regional PiB retention

A voxel-based statistical analysis was performed using the Statistical Parametric Mapping program, Version 2 (SPM2), and Matlab 6.5 for Windows (MathWorks, Natick, MA). Spatial registration of the ^{11}C-PiB PET (normalized to cerebellar ROI) was performed based on spatial normalization parameters estimated on the basis of MRI T1 images. To this end, individual MRIs were co-registered to ^{11}C-PiB PET/CT images and the co-registered MRIs were normalized to the MNI152 T1 template implemented in the SPM2 package. These spatial normalization parameters were then applied to the ^{11}C-PiB PET images, which were subsequently smoothed using a 12 mm Gaussian kernel. An SPM regression analysis was performed without global normalization, since the ^{11}C-PiB PET images had been normalized to the cerebellar ROI PiB binding. We defined statistical significance as a false discovery rate (FDR) corrected $p < 0.05$ and at a cluster extent threshold of 150 voxels.

eReferences

1. Sachdev P, Wen W, Shnier R, Brodaty H. Cerebral blood volume in T2-weighted white matter hyperintensities using exogenous contrast based perfusion MRI. *J Neuropsychiatry Clin Neurosci*. 2004;16(1):83-92.
2. Jeon S, Yoon U, Park JS, et al. Fully automated pipeline for quantification and localization of white matter hyperintensity in brain magnetic resonance image. *International Journal of Imaging Systems and Technology*. 2011;21(2):193-200.

