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ORIGINAL ARTICLE

Impact of Metabolic Activity of Vertebra and Amygdala on Stroke Recurrence: A Prospective Cohort Study

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BACKGROUND: Elevated metabolic activity of amygdala is known to be related to atherosclerotic cardiovascular event by increasing inflammatory cell production from bone marrow. We tried to identify the factors of metabolic activity in the amygdala, vertebrae, liver, spleen, and internal carotid artery related to the future vascular events after stroke.

METHODS: A total of 110 patients with acute stroke were included (72±10 years of age, 39% women) and underwent whole-body ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography between August 1, 2015 and February 28, 2020. We compared the FDG uptake in the amygdala, vertebrae, liver, spleen, and internal carotid artery between patients with and without recurrent vascular event. Cox proportional hazards model was used to identify factors related to recurrent stroke and vascular event.

RESULTS: During the median follow-up period of 18 months, 22 patients experienced vascular events, including 15 stroke recurrence. Patients with recurred vascular event had a significantly higher FDG uptake in the amygdala and vertebrae than those without. The Cox proportional hazard model including diabetes, renal function, and carotid stenosis showed that a higher FDG uptake in the amygdala was independently associated with total vascular events (hazard ratio, 3.11 [95% CI, 1.11–8.70]) and higher FDG uptake in the vertebrae with stroke recurrence (hazard ratio, 4.94 [95% CI, 1.29–18.9]).

CONCLUSIONS: The increased metabolic activities of the vertebrae and amygdala are related to future vascular event among stroke survivors.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: amygdala ■ bone and bones ■ metabolism ■ positron emission tomography ■ stroke

See Editorial by Seligowski and Tawakol

Atherosclerosis is a systemic disorder involving medium- to large-sized arterial walls, mediated by lipid deposition and subsequent inflammation, resulting in plaque rupture and thrombus formation. The treatment strategy is targeted to the conventional vascular risk factors such as dyslipidemia, hypertension, and diabetes; however, a considerable number of patients still experience vascular events even after the achievement of optimal aforementioned risk factor control.[1] Preclinical and clinical studies have shown that inflammation is a key factor in atherosclerosis progression and plaque rupture, and several recent clinical trials investigating anti-inflammatory therapeutic strategies have shown promising effects in reducing vascular events independent of lipid profile management.[2,3] Insulin resistance is another modifiable risk factor of vascular disease that is prevalent among patients with stroke.[4] Understanding the mechanism of less appreciated, but important risk factors on atherosclerosis is important to prevent future vascular event among stroke survivors.

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CLINICAL PERSPECTIVE

Elevated metabolic activities of amygdala and vertebrae are known to be related to atherosclerotic cardiovascular event by increasing inflammatory cell production and recruitment. In this study, the impact of metabolic activities of amygdalae and vertebrae on future vascular events were prospectively investigated among patients with stroke by applying ^{18}F-fluorodeoxyglucose positron emission tomography. We found that the patients with elevated glucose uptake in the vertebrae and amygdala were associated with increased risk of future vascular events, possibly by metabolic dyshomeostasis.

Nonstandard Abbreviations and Acronyms

BMI	body mass index
MLR	monocyte lymphocyte ratio
NIHSS	National Institute of Health stroke scale
SUVmax	the maximum standardized uptake value
SUVmean	the mean standardized uptake value
TOAST	Trial of Org 10172 in Acute Stroke Treatment

Recent studies have shown that hematopoietic organs, such as the spleen and vertebral bodies, have crucial roles in atherosclerosis progression by supplying inflammatory cells.[5,6] These activated hematopoietic organs have shown increased uptake of glucose, and its uptake intensities from ^{18}F-fluorodeoxyglucose positron emission tomography (FDG PET) were associated with systemic inflammatory markers and future vascular events among patients with coronary atherosclerosis.[6] The metabolic activity of the amygdala is known to represent an emotional response to fear and anxiety and increased FDG uptake in this area is associated with future vascular events because it is linked to an augmented arterial inflammation and inflammatory cell production from the bone marrow via the sympathetic nervous system.[7] Patients with stroke are generally older than those with coronary artery diseases and have more heterogeneous mechanism involved, but the role of amygdala and hematopoietic organ activity had not been reported among stroke survivors. We investigated the impact of the metabolic activities of the amygdala and vertebrae on stroke recurrence and future vascular events among patients with stroke.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Patient Inclusion

The CARPET (Cerebral Atherosclerosis Research with Positron Emission Tomography) is a prospective registry to understand the pathophysiology of cerebral atherosclerosis by applying FDG PET in patients with acute cerebral infarction. This study was reviewed and approved by the institutional review board of Chung-Ang University Hospital (C2015061), and informed consent was obtained from every patient according to the Declaration of Helsinki. All eligible patients underwent brain computed tomography angiography at admission and we included patients with carotid atherosclerosis of ≥ 50% from brain computed tomography angiography. To understand the relationship between cerebral atherosclerosis burden and hematopoietic organ activities, we also included patients with stroke with mild carotid stenosis or without carotid atherosclerosis during the same inclusion period. We excluded patients with overt cancer or autoimmune diseases, advanced renal impairment with an estimated glomerular filtration rate <30 mL/(min·1·73m^2), uncontrolled diabetes, or other unstable medical conditions. The enrolled patients underwent a comprehensive stroke etiology workup including brain magnetic resonance imaging, cardiac evaluation, and bone mineral density evaluation with dual-energy X-ray absorptiometry. Blood tests included common blood counts with differential count of major inflammatory cell subtypes; renal/liver function tests; lipid profiles including total cholesterol, high-/low-density lipoprotein cholesterol, and lipoprotein a levels; fasting and postprandial insulin and C-peptide levels; bone metabolism markers, including parathyroid hormone, vitamin D, and osteocalcin levels; coagulation profile, highly sensitive C-reactive protein, and homocysteine levels.

FDG PET Protocol and Image Analysis

Once a patient was stabilized after an index stroke, whole-body FDG PET and CT were conducted with a combined scanner (Gemini TF 16, Philips Medical Systems, Cleveland, OH) after overnight fasting by a standard protocol as described below. Approximately 60 minutes after the intravenous injection of 259 to 370 MBq (7–10 mCi) of FDG, PET images were acquired for 5 min/bed for the head and 1 min/bed from the skull base to the proximal thigh (120 kVp, 50 mA). Two nuclear medicine specialists (J.W.S. and R.L.) blinded to the clinical information assessed the FDG uptake in each organ. The mean standardized uptake values (SUVmean) of the solid organs were measured in the liver, spleen, and third to fifth lumbar vertebrae as described previously.[7,8] Briefly, FDG uptake was measured in the lumbar vertebrae by placing a region of interest within individual vertebra, and the highest mean SUVs were selected to derive mean FDG uptake of third to fifth lumbar vertebrae.[7,8] The maximal target-to-background activity of the carotid artery was derived by dividing the maximum SUV (SUVmax) of the proximal internal carotid artery at the level with the largest atheroma by the SUVmean of the ascending aorta blood, as described previously.[9,10] The maximal target-to-background ratio of carotid artery was also derived with the references of the SUVmean of internal jugular vein at the same level and the SUVmean of blood pool in the superior vena cava.[11] The maximal target-to-background ratio of the amygdala was calculated by dividing the SUVmax of the amygdala by the SUVmean of the inferior temporal lobe. The maximal target-to-background

ratio of the amygdala was also calculated with a reference to the SUVmean of both cerebellum for the sensitivity analysis. Since amygdala is a small structure within limbic system, we also applied statistical parametric mapping with following protocol: Images were reconstructed with vendor provided iterative time-of-flight reconstruction algorithm (BLOB-OS-TF, with 3 iterations/33 subsets) and the reconstructed matrix size was 144×144 with resulting dimension of voxels of 4×4 mm, followed by spatially normalized to Montreal Neurological Institute space using SPM8 (University College of London, United Kingdom) and additional smoothing with an 8-mm Gaussian filter. We defined voxel of interest of amygdala and extracted intensity of FDG using MRICron (https://www.nitrc.org/projects/mricron) with automated anatomical labeling map.

Patient Follow-Up

The enrolled patients were categorized into 4 groups according to the carotid atherosclerosis burden: group 1, patients with stroke with symptomatic carotid atherosclerosis of ≥ 50%; group 2, patients with stroke with nonculprit carotid atherosclerosis of ≥ 50%; group 3, patients with stroke with mild carotid stenosis <50%; and group 4, patients with stroke without carotid stenosis. The patients were grouped according to the stroke mechanism of TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification including (1) large-artery atherosclerosis, (2) cardioembolism, (3) small-vessel occlusion, (4) other determined etiology, (5) undetermined etiology, and (6) transient ischemic attack.[12] The patients were also categorized according to the initial infarct size in the brain MRI: (1) no lesion (transient ischemic attack), (2) single and small lesion within 20 mm, and (3) multiple or large lesion (>20 mm). All included patients received the best medical treatment according to the recent stroke management guideline, including either high-intensity statin for atherosclerotic patients with stroke or moderate-intensity statin for others. After discharge, the patients were followed every 2 to 3 months at outpatient clinic to detect any vascular event including stroke recurrence, myocardial infarction, peripheral artery disease, or death. When a patient could not visit the outpatient clinic, information was requested from the most reliable caregiver.

Statistical Analyses

We first compared the clinical and laboratory variables according to the carotid atherosclerosis burden to understand the relationship between carotid atherosclerosis and FDG uptake in the various organs. As a sensitivity analysis, the FDG uptake level at each organ was also compared according to TOAST stroke mechanism and infarct size. The continuous variables were expressed as mean±standard deviations and compared by the analysis of variance or Kruskal-Wallis test. Bonferroni comparison was used as post hoc analysis if necessary. The categorical variables were expressed as numbers of patients with percentage and compared by χ^2 test. Spearman correlation analysis was performed between FDG uptake levels at different organs and laboratory variables. Second, we compared the clinical, laboratory, and imaging variables between patients with and without stroke recurrence to find the prognostic factors predicting future vascular event. Continuous variables were compared by using Student *t* test or Mann-Whitney *U* test and categorical variables by χ^2 test or Fisher exact test. Since we found that patients with recurrent stroke had a significantly higher FDG uptake in the amygdala and vertebrae, the patients were dichotomized by cutoff points at each organ derived from the receiver operating characteristic curve analysis and the Youden index with the highest discrimination power. Kaplan-Meier survival curves for the stroke recurrence and overall vascular event were compared by log-rank tests. Cox proportional hazard models were used to assess the association of FDG uptake in the amygdala or vertebrae on stroke recurrence or overall future vascular event adjusting for potential confounders derived from bivariate analyses with P values <0.05. The date of FDG PET taken was defined as time 0 in the time-to-event analyses. Statistical significance was determined at a P<0.05. All analyses were performed by SPSS software (version 23.0, IBM Corp, Armonk, NY).

RESULTS

A total of 110 patients were included in the study between August 1, 2015 and February 28, 2020 (mean age: 72±10 years, 39% women). The number of patients with symptomatic carotid atherosclerosis was 28; another 29 patients had moderate to severe nonculprit carotid stenosis with other stroke sources, including 19 with intracranial atherosclerosis and 10 with cardioembolism. The clinical characteristics and FDG uptake levels in various organs were compared according to the carotid atherosclerosis burden (Table 1). Patients with symptomatic carotid atherosclerosis had the highest FDG uptake levels in the proximal internal carotid artery. The FDG uptake in the vertebrae and the liver tended to be lower among patients with symptomatic carotid atherosclerosis than those with mild carotid stenosis. The FDG uptakes in the spleen or amygdala were not statistically different among the 4 groups. The mean time interval between index stroke and FDG PET image was 17.3±12.5 days, and there was negative correlation between the time interval and FDG uptake in the carotid artery (Spearman ρ=−0.194, P=0.042). The relation between time interval and metabolic activity in the amygdala (Spearman ρ=0.027, P=0.78) or vertebrae (Spearman ρ=0.106, P=0.27) was not significant. There was no significant relationship between FDG uptake at each organ and stroke mechanism (Table S1) or infarct size (Table S2).

During the median follow-up period of 18 months, 15 patients experienced stroke recurrence. The recurrent stroke mechanism included atherosclerosis (n=7, 6 intracranial atherosclerosis and 1 extracranial carotid stenosis), cardioembolism due to new atrial fibrillation (n=4), miscellaneous etiologies (n=2, one essential thrombocythemia and the other new cancer-related coagulation disorder), cerebellar hemorrhage (n=1), and unknown etiology (n=1). Another 7 patients experienced vascular events other than stroke, including 4 deaths, 2 cases of coronary artery disease, one transient ischemic attack, and 1 case of iliac artery occlusion requiring embolectomy. When the

Table 1. Comparison of Clinical and Laboratory Characteristics According to Carotid Stenosis

	Group 1 (n=28)	Group 2 (n=29)	Group 3 (n=26)	Group 4 (n=27)	*P* value*
Age, y, mean (SD)	73.0 (8.9)	75.6 (7.4)	69.4 (11.2)	70.1 (11.3)	0.08
Sex, female, n (%)	8 (28.6)	13 (44.8)	10 (38.5)	12 (44.4)	0.32
Hypertension, n (%)	25 (89.3)	25 (86.2)	19 (73.1)	23 (85.2)	0.42
Diabetes, n (%)	14 (50.0)	16 (55.2)	13 (50.0)	8 (29.6)	0.12
Atrial fibrillation, n (%)	4 (14.3)	10 (34.5)	4 (15.4)	13 (48.1)	0.03
Coronary artery disease, n (%)	5 (17.9)	1 (3.4)	3 (11.5)	3 (11.1)	0.65
Previous stroke, n (%)	3 (10.7)	9 (31.0)	2 (7.7)	5 (18.5)	0.99
Body mass index, kg/m^2, mean (SD)	22.8 (3.4)	22.5 (3.2)	23.1 (3.2)	24.1 (3.4)	0.29
Initial NIHSS, mean (SD)	3.8 (4.6)†	6.2 (6.3)	7.3 (8.3)	10.4 (8.0)†	0.007
White blood cell count, ×10^9/L, mean (SD)	6.8 (1.4)	8.0 (2.4)	8.0 (2.7)	7.6 (2.5)	0.18
MLR, mean (SD)	0.37 (0.22)	0.47 (0.53)	0.34 (0.17)	0.28 (0.16)	0.21
Hemoglobin, g/dL, mean (SD)	12.8 (2.2)	12.9 (2.0)	13.2 (2.1)	13.4 (2.0)	0.66
Platelet count, ×10^9/L, mean (SD)	225 (74)	240 (66)	267 (74)	254 (99)	0.23
hsCRP, mg/dL, mean (SD)	5.1 (12.1)	9.6 (20.2)	8.6 (19.3)	5.8 (15.9)	0.72
LDL cholesterol, mg/dL, mean (SD)	98.1 (47.8)	89.6 (32.2)	95.1 (23.7)	92.1 (24.6)	0.80
eGFR, mL/(min·1.73m^2), mean (SD)	84.4 (24.6)	78.0 (23.8)†	91.8 (28.4)	100.6 (32.6)†	0.02
HbA1c, %, mean (SD)	6.2 (1.1)	6.5 (1.1)	6.4 (1.2)	6.2 (1.3)	0.70
Carotid stenosis %, mean (SD)	73.8 (19.7)†	64.7 (17.4)†	24.8 (13.0)†	0.0 (0.0)†	<0.001
TBRmax at carotid artery, mean (SD)	1.08 (0.26)†	1.03 (0.17)	0.96 (0.17)	0.92 (0.16)†	0.01
SUVmean spleen, mean (SD)	1.50 (0.23)	1.59 (0.39)	1.62 (0.23)	1.56 (0.25)	0.45
SUVmean liver, mean (SD)	1.64 (0.26)	1.73 (0.39)	1.87 (0.31)	1.73 (0.29)	0.07
SUVmean, 3–5th lumbar vertebrae, mean (SD)	1.09 (0.23)	1.13 (0.30)	1.28 (0.32)	1.20 (0.34)	0.09
TBRmax at amygdala, mean (SD)	1.02 (0.15)	1.04 (0.13)	1.00 (0.11)	1.02 (0.08)	0.78

Group 1, patients with stroke with symptomatic carotid atherosclerosis of ≥50%; group 2, patients with stroke with nonculprit carotid atherosclerosis of ≥50%; group 3, patients with stroke with mild carotid stenosis <50%; and group 4, patients with stroke without carotid stenosis. eGFR indicates estimated glomerular filtration rate; GFR glomerular filtration rate; HbA1c glycated hemoglobin A1c; hsCRP, highly sensitive C-reactive protein; LDL, low-density lipoprotein; MLR, monocyte to lymphocyte ratio; NIHSS, National Institute of Health stroke scale; SUVmean, the mean of standardized uptake value; and TBRmax, the maximum value of target-to-background ratio.

*P values from the analysis of variance or Kruskal-Wallis test for continuous variable or from χ^2 test for the categorical variables.

†P<0.05 by post hoc Bonferroni correction in comparison to group 4.

clinical and laboratory variables were compared between the patients with and without stroke recurrence, those with a recurrent stroke had a significantly higher proportion of diabetes and a higher degree of carotid stenosis, but lower estimated glomerular filtration rate than those with stable prognosis (Table 2). The patients with recurrent stroke had a significantly higher FDG uptake in the lumbar vertebrae (1.14±0.28 versus 1.36±0.40, P=0.009, Mann-Whitney U test) and amygdala (1.01±0.11 versus 1.09±0.14, P=0.017, Mann-Whitney U test) than those without (Table 2). The representative images showed that a stroke patient without elevated metabolic activities in the amygdala and the vertebrae did not experience any additional vascular event (Figure 1A). However, another patient with elevated FDG uptake in the vertebrae and amygdala experienced recurrent stroke involving the right parietal cortex after 1 year, the left pons after 2 years, and finally, a coronary event leading to death after 3 years (Figure 1B). When the glucose uptake in the amygdala on the reference of inferior temporal lobe were compared between the patients with and without overall vascular event, those with recurrent vascular event had a significantly higher FDG uptake (1.00±0.11 versus 1.09±0.12, P=0.003, Mann-Whitney U test) than those without (Figure 1C). The comparison by TBR of glucose uptake on the reference of cerebellum also showed elevated glucose uptake among the patients with vascular event (0.87±0.95 versus 0.92±0.12, P=0.003, Mann-Whitney U test, Figure 1C). The analysis with statistical parametric mapping on the reference of temporal pole (D, 1.14±0.11 versus 1.20±0.11, P=0.029, Mann-Whitney U test) and cerebellum (D, 1.08±0.12 versus 1.16±0.12, P=0.011, Mann-Whitney U test) consistently showed that glucose uptake in the amygdala was elevated among those with recurrent vascular event.

The Spearman correlation analysis between FDG uptake in various organs and clinical variables disclosed that FDG uptake in the vertebrae correlated with the body mass index (Spearman ρ=0.320, P=0.001), triglyceride (Spearman ρ=0.223, P=0.019), fasting C-peptide level (Spearman ρ=0.222, P=0.026), bone mineral density (Spearman ρ=0.213, P=0.028), FDG uptake in the liver (Spearman ρ=0.453, $P < 0.001$) and the spleen (Spearman ρ=0.443, P<0.001), but negatively correlated with age (Spearman ρ=−0.192, P=0.045; Figure 2A). The FDG uptake in the amygdala correlated

Table 2. Factors Associated With Stroke Recurrence

	Patients without recurrence (n=95)	Patients with recurrence (n=15)	*P* value
Age, y, mean (SD)	72.7 (9.8)	68.9 (10.7)	0.17
Sex, female, n (%)	40 (42.1)	3 (20.0)	0.10
Hypertension, n (%)	78 (82.1)	14 (93.3)	0.28
Diabetes, n (%)	40 (42.1)	11 (73.3)	0.02
Atrial fibrillation, n (%)	27 (28.4)	4 (26.7)	0.89
Previous stroke, n (%)	14 (14.7)	5 (33.3)	0.08
Initial NIHSS, mean (SD)	7.3 (7.5)	3.9 (4.3)	0.09
SBP, mmHg, mean (SD)	153 (28)	148 (24)	0.56
White blood cell count, x 10^9/L, mean (SD)	7.6 (2.3)	7.3 (2.9)	0.61
MLR, mean (SD)	0.35 (0.21)	0.50 (0.70)	0.09
Hemoglobin, g/dL, mean (SD)	12.9 (2.0)	13.8 (2.0)	0.12
Platelet count, ×10^9/L, mean (SD)	244 (73)	260 (113)	0.47
hsCRP, mg/dL, mean (SD)	7.9 (18.2)	3.5 (5.4)	0.37
Total cholesterol, mg/dL, mean (SD)	155.5 (44.6)	160.9 (37.2)	0.66
LDL cholesterol, mg/dL, mean (SD)	92.7 (28.5)	100.1 (38.2)	0.42
Triglyceride, mg/dL, mean (SD)	117.4 (91.5)	156.7 (101.2)	0.13
eGFR, mL/(min·1.73m^2), mean (SD)	91.1 (28.5)	71.8 (21.9)	0.01
HbA1c, %, mean (SD)	6.3 (1.1)	6.7 (1.3)	0.15
BMD T score at femur, mean (SD)	−1.26 (1.54)	−0.81 (1.31)	0.28
Carotid stenosis %, mean (SD)	38.7 (32.8)	60.8 (31.7)	0.02
Dual antiplatelet therapy, n (%)	51 (53.7)	10 (66.7)	0.35
High-intensity statin, n (%)	55 (57.9)	7 (46.7)	0.42
TBRmax at carotid artery, mean (SD) aortic pool as reference	0.99 (0.21)	1.06 (0.14)	0.24
TBRmax at carotid artery, mean (SD) IJV as reference	1.22 (0.25)	1.26 (0.30)	0.32
TBRmax at carotid artery, mean (SD) SVC as reference	1.07 (0.23)	1.12 (0.20)	0.47
SUVmean spleen, mean (SD)	1.56 (0.29)	1.61 (0.23)	0.52
SUVmean liver, mean (SD)	1.73 (0.33)	1.79 (0.26)	0.51
SUVmean, 3–5th lumbar vertebrae, mean (SD)	1.14 (0.28)	1.36 (0.40)	0.009
TBRmax amygdala, mean (SD)	1.01 (0.11)	1.09 (0.14)	0.02

P values were driven by Student *t* test or Mann-Whitney *U* test for continuous variables or by χ^2 test or Fisher exact test for categorical variables. BMD indicates bone mineral density from dual-energy X-ray densitometry; eGFR, estimated glomerular filtration rate; HbA1c glycated hemoglobin A1c; hsCRP, highly sensitive C-reactive protein; IJV, internal jugular vein; LDL, low-density lipoprotein; MLR, monocyte to lymphocyte ratio; NIHSS, National Institute of Health stroke scale; SBP, systolic blood pressure; SUVmean, the mean of standardized uptake value; SVC, superior vena cava; and TBRmax, the maximum value of target-to-background ratio.

with triglyceride (Spearman ρ=0.196, *P*=0.040) and negatively correlated with FDG uptake in the liver (Spearman ρ=−0.213, *P*=0.026) but had no association with FDG uptake in the vertebrae (Spearman ρ=−0.035, *P*=0.718). The FDG uptake in the carotid artery correlated with the degree of stenosis from computed tomography angiography (Spearman ρ=0.258, *P*=0.006) but did not correlate with FDG uptake in the vertebrae or amygdala. When we analyzed the receiver operating characteristic curve to predict future stroke recurrences, the cutoff values were 1.14 (area under the curve=0.661, sensitivity=73.3%, specificity=56.8%) for vertebral body and 1.03 (area under the curve=0.686, sensitivity=80.0%, specificity=62.1%) for amygdala. The patient distribution pattern in terms of FDG uptake levels in the amygdala (*x* axis) and the vertebrae (*y* axis) revealed that patients with stroke recurrence (red dot) and other vascular events or death (orange dot) were concentrated on the quadrant with a higher FDG uptake in both organs (Figure 2B).

Next, we tested whether an increased FDG uptake in the vertebrae and amygdala was independently associated with future vascular events. The Kaplan-Meier survival analysis demonstrated that patients with a high FDG uptake in the vertebral body (red line) were associated with a shorter stroke recurrence-free survival (log-rank test, *P*=0.009, Figure 3A), and with overall vascular events and death-free survival (log-rank test, *P*=0.016,

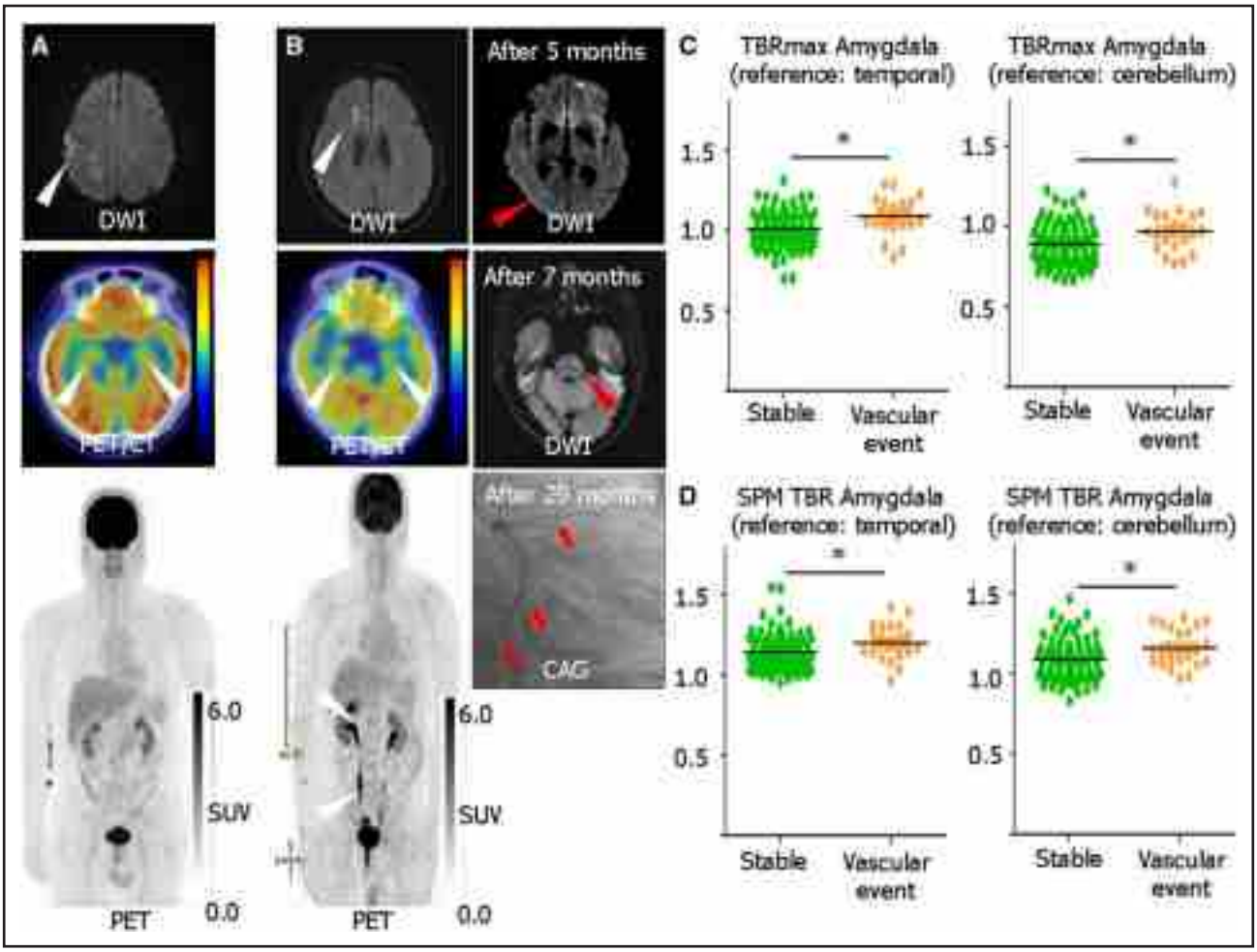

Figure 1. Representative ^{18}F-fluorodeoxyglucose positron emission tomography (FDG PET) images of patients with and without recurrent stroke.

The patient with right middle cerebral artery territory infarction (**A**) had normal glucose uptake in the amygdala from FDG PET (white arrowheads) and did not experience vascular event after index stroke. Another patient with right frontal lobe infarction (**B**) had increased FDG uptake in the amygdala and vertebral bodies (white arrowheads). The patient experienced 2 episodes of stroke recurrence involving the right parietal cortex one year after index stroke and left pons (red arrowheads) after 2 years and died of cardiac arrest due to coronary disease (red arrows) after 3 years. The glucose uptake in the amygdala was significantly higher among the patients with additional vascular event after index stroke, when compared by the target-to-background ratio on the reference of glucose uptake in the inferior temporal lobe (C, 1.00±0.11 versus 1.09±0.12, *P*=0.003, Mann-Whitney U test) or cerebellum (C, 0.87±0.95 versus 0.92±0.12, *P*=0.003, Mann-Whitney *U* test). The analysis with statistical parametric mapping also demonstrated that the glucose uptake in the amygdala was elevated among the patients with recurrent vascular event on the reference of inferior temporal lobe (D, 1.14±0.11 versus 1.20±0.11, *P*=0.029, Mann-Whitney *U* test) and cerebellum (D, 1.08±0.12 versus 1.16±0.12, *P*=0.011, Mann-Whitney *U* test). CAG indicates coronary angiography; DWI, diffusion-weighted image; PET/CT, positron emission tomography/computed tomography; SPM, statistical parametric mapping; SUV, standardized uptake value; and TBR, target-to-background ratio.

Figure 3B) than those with a low FDG uptake in the vertebrae (blue line). Patients with an increased FDG uptake in the amygdala (orange line) also had a shorter stroke recurrence-free survival (log-rank test, *P*=0.036, Figure 3C) and with overall vascular event and death-free survival (log-rank test, *P*=0.031, Figure 3D) than those with a low FDG uptake in the amygdala (green line). Cox proportional hazard model including diabetes, estimated glomerular filtration rate, and carotid stenosis demonstrated that the FDG uptake in the vertebrae was significantly associated with future stroke recurrence (hazard ratio, 4.94 [95% CI, 1.29–18.9]; *P*=0.020, Table 3). Regarding overall vascular events and death, FDG uptake in the amygdala was a significant predictor after adjusting clinical variables (hazard ratio, 3.11 [95% CI, 1.11–8.70]; *P*=0.031, Table 3).

DISCUSSION

Patients with stroke with symptomatic carotid atherosclerosis were found to have the highest level of FDG uptake in the carotid wall, but it was not related to stroke recurrence due to heterogeneous mechanisms of recurrence. The patients with stroke with increased FDG uptake in

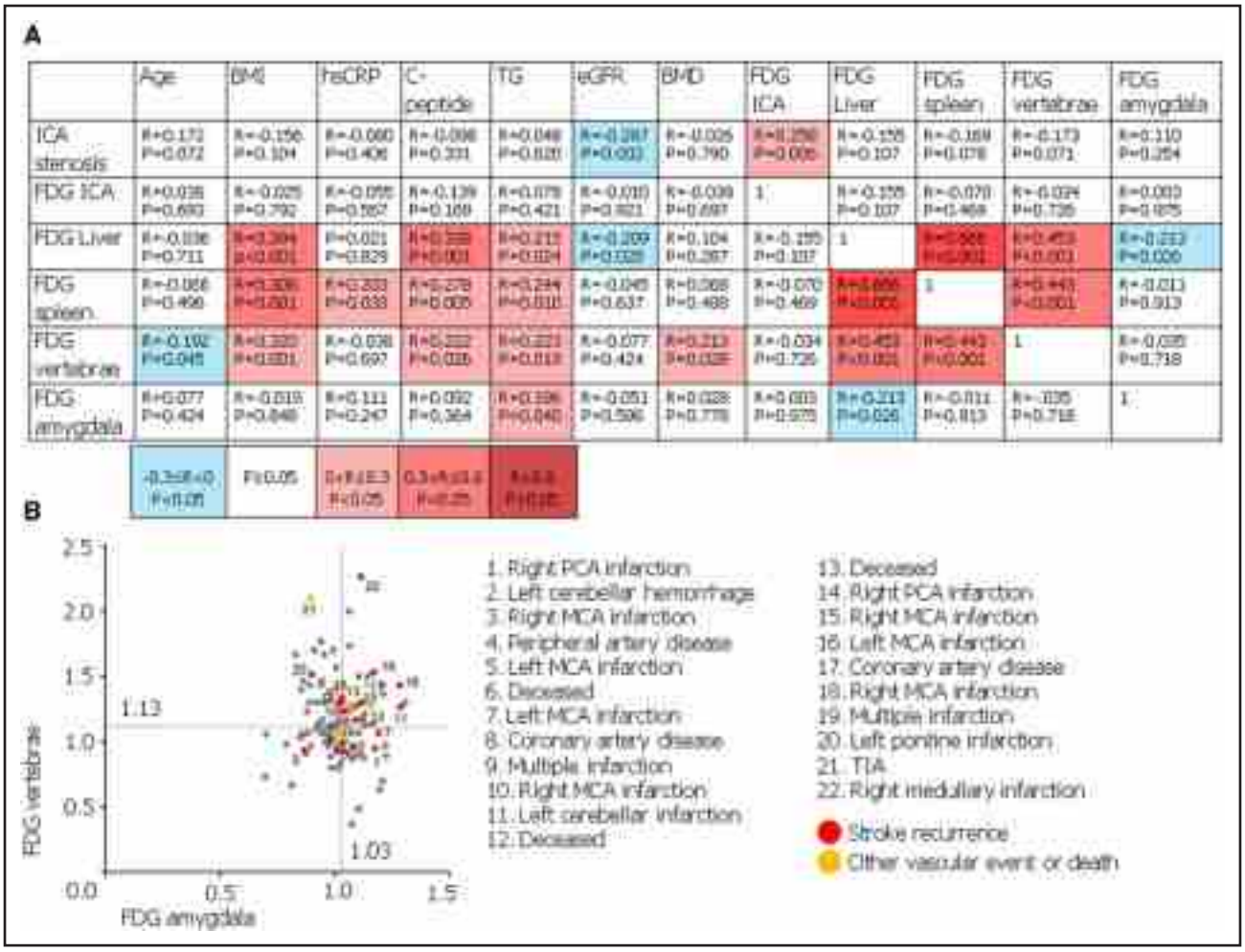

Figure 2. Correlation analysis between ^{18}F-fluorodeoxyglucose (FDG) uptake in various organs and laboratory variables. Correlation analysis (**A**) disclosed that the FDG uptake level in the lumbar vertebrae is associated with the body mass index (BMI, Spearman ρ=0.320, *P*=0.001), C-peptide level (Spearman ρ=0.222, *P*=0.026), triglyceride (Spearman ρ=0.223, *P*=0.019), bone mineral density (r=0.213, *P*=0.028), FDG uptake in the liver (Spearman ρ=0.453, *P*<0.001) and spleen (Spearman ρ=0.443, *P*<0.001), but negatively correlated with age (Spearman ρ=−0.192, *P*=0.045). The uptake level of FDG in the amygdala is positively correlated with triglyceride (Spearman ρ=0.196, *P*=0.040) and inversely correlated with FDG uptake in the liver (Spearman ρ=−0.213, *P*=0.026). Although there was no correlation between FDG uptake in the vertebrae and the amygdala (r=−0.035, *P*=0.718, **B**), most patients with recurrent stroke (red circle) and other vascular events or death (yellow circle) tended to be located in the zone with elevated FDG uptake of >1.03 in the amygdala and >1.13 in the vertebrae (**B**). BMD indicates bone mineral density; eGFR, estimated glomerular filtration rate; hsCRP, highly sensitive C-reactive protein; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; TG, triglyceride; and TIA, transient ischemic attack.

the lumbar vertebrae or amygdala had frequent stroke recurrence and overall vascular events. The vertebral glucose uptake was found to be related to body mass index, triglyceride, fasting C-peptide level, and bone mineral density. The glucose uptake in the amygdala correlated with triglyceride.

Recent studies have shown that the activated amygdala is closely related to FDG uptake in the arterial wall and hematopoietic organs, elucidating the role of stress-induced inflammation in developing future atherosclerotic cardiovascular event.[7] Although FDG uptake in the amygdala was independently related with future vascular event, its association with FDG uptake in the vertebrae and carotid artery was neutral among patients with stroke. The positive association between amygdala glucose uptake and serum triglyceride may suggest the role of limbic system on systemic lipid metabolism among patients with stroke. Observational studies have demonstrated that elevated triglyceride is closely related with intracranial atherosclerosis and atrial fibrillation.[13,14] Poststroke cardiovascular complication due to brain-heart neuronal and humoral interaction could be another mechanism of future vascular event after stroke.[15] Accordingly, the mechanisms of recurrent stroke in this study were diverse, including intracranial atherosclerosis in 6 patients and new-onset atrial fibrillation in 4 patients, and only one patient experienced vascular event due to carotid atherosclerosis. The inverse relationship between FDG uptake in the amygdala and liver is another interesting finding which requires further investigation. Liver

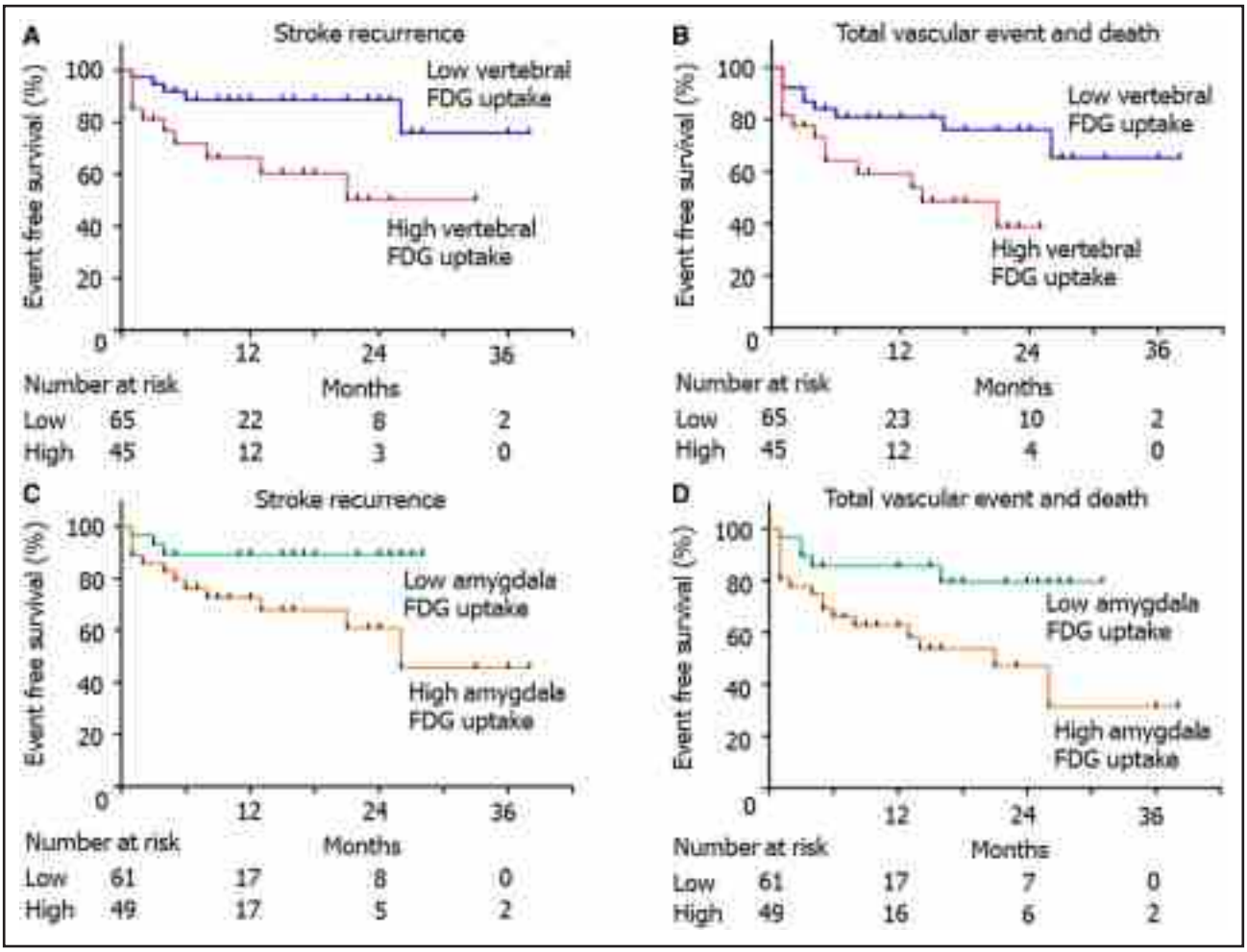

Figure 3. Survival curve analysis according to the ^{18}F-fluorodeoxyglucose (FDG) uptake in the lumbar vertebrae and the amygdala.

The Kaplan-Meier survival curve analysis demonstrates that patients with elevated FDG uptake in the vertebral body (red line, cutoff of >1.13) had a shorter stroke recurrence-free survival (log-rank test, *P*=0.009, **A**), and overall vascular events and death-free survival (log-rank test, *P*=0.016, **B**) than those with a low vertebral FDG uptake (blue line). Patients with an increased FDG uptake in the amygdala (orange line, cutoff of >1.03) also had a shorter stroke recurrence-free survival (log-rank test, *P*=0.036, **C**) and overall vascular event-free survival (log-rank test, *P*=0.031, **D**) than those with a low FDG uptake in the amygdala (green line).

is a vital organ for cholesterol homeostasis and protein synthesis regulating inflammation and coagulation cascade, and several observational studies have reported that nonalcoholic fatty liver disease or combined liver fibrosis is associated with subclinical atherosclerosis and vascular event.[16,17]

Patients with stroke with an increased FDG uptake in the vertebrae had more frequent stroke recurrence. Studies from coronary artery disease patients have found that increased FDG uptake in the vertebrae is associated with elevated serum C-reactive protein, arterial FDG uptake, and future vascular event.[6] However, the

Table 3. Multivariable Cox Proportional Regression for Stroke Recurrence and Overall Vascular Event

	Stroke recurrence			Overall vascular event and death		
	Hazard ratio	CI	*P* value	Hazard ratio	CI	*P* value
Diabetes	2.68	0.74–9.73	0.13	2.55	0.98–6.66	0.06
Estimated GFR	0.99	0.97–1.01	0.41	1.01	0.99–1.02	0.52
Carotid stenosis %	1.02	0.99–1.04	0.08	1.01	0.99–1.02	0.52
Higher FDG at vertebra	4.94	1.29–18.9	0.02	2.50	0.98–6.37	0.05
Higher FDG at amygdala	3.00	0.82–10.9	0.09	3.11	1.11–8.70	0.03

GFR indicates glomerular filtration rate; and FDG, ^{18}F-fluorodeoxyglucose.

FDG uptake in the vertebrae of stroke survivors did not correlate with systemic inflammatory markers or FDG uptake in the carotid artery. The interpretation of FDG uptake in the vertebrae might not solely depend on the hematopoietic activity, and rather represent the activity of heterogeneous cellular components within the skeletal microenvironment, including osteoblast, stromal cells such as bone marrow adipocyte, and mesenchymal/hematopoietic stem cells.[18] Observational and preclinical studies have reported that aging process is associated with decreased osteogenesis and hematopoiesis, and increased adipogenesis within bone.[19] Our group reported that reduced bone mineral density is independently associated with cerebral small vessel disease burden among patients with stroke, possibly by defective mineral homeostasis and hematopoiesis.[20] A recent study demonstrated that the bone marrow adipose tissue has a distinct metabolic phenotype with decreased insulin responsiveness, but higher glucose uptake to cold stimuli, as compared to adipose tissues from other body parts.[21] Considering the positive association between C-peptide level and FDG uptake in the vertebrae, it is conceivable that glucose uptake in vertebrae may reflect insulin resistance of stroke survivors, which is related to future vascular event. It is also possible that coexisting age-related bone degeneration might have attenuated FDG uptake intensity due to inflammatory cell production after stroke because the vertebral FDG uptake was correlated with bone mineral density and inversely correlated with age. Previous study showed that the patients with stroke had a significantly lower level of FDG uptake in the vertebrae than control subjects, and the vertebral FDG level inversely correlated with FDG uptake in the carotid artery.[8]

Several limitations exist in this study. First, this prospective cohort was based on a single university hospital with relatively small number of patients and events. Therefore Cox proportional hazard model could be underpowered to detect factors associated with future vascular events. However, we could perform in-depth analyses to elucidate stroke mechanism in every patient and the biological significance of metabolic activity of each organ from FDG PET could have been analyzed based on detailed laboratory studies. The metabolic activity of the amygdala had been reported to be influenced by stress and anxiety, but we did not assess the emotional status of the included patients because of neurological deficit following stroke. Amygdala is a small region located at the tip of the temporal lobe and is supplied by the anterior choroidal artery. Although the patients in this study did not have a stroke involving this limbic structure, it is possible that the glucose uptake level in the amygdala be influenced by the location or the size of cerebral infarction. Regional blood-brain barrier disruption or inflammatory cell infiltration may also affect FDG uptake in this small region. The impact of the metabolic activity in the amygdala and vertebrae on stroke recurrence requires external validation considering small differences in absolute uptake values in the 2 structures and relatively high vascular event rate. Whether elevated metabolic activity of amygdala or vertebrae could be a therapeutic target of vascular disease prevention requires further investigation. Among patients with depression following acute coronary syndrome, treatment with escitalopram, a selective serotonin reuptake inhibitor, significantly lowered the risk of major cardiac events.[22]

The glucose uptake status in the amygdala and bone could help to predict stroke recurrence and vascular events among patients with stroke. The metabolic activity of amygdala and vertebrae may affect thromboembolic event via metabolic dyshomeostasis. Further studies are necessary to elucidate the distinct pathophysiological links between metabolic activities in these specific regions and vascular events, and therapeutic strategies based on novel mechanisms.

ARTICLE INFORMATION

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J.-M. Kim and Dr Park contributed to conception and design of the study. J.-M. Kim, R. Lee, Y. Kim, Dr Jeong, E.S. Lee, H.R. Kim, and Dr Seok participated in acquisition and analysis of data. J.M. Kim, R. Lee, H.R. Kim, Drs Seok, and Park drafted a significant portion of the article or figures.

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Disclosures

None.

Supplemental Material

Tables S1 and S2

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