# Postoperative Increase in Cerebral White Matter Fractional Anisotropy on Diffusion Tensor Magnetic Resonance Imaging Is Associated With Cognitive Improvement After Uncomplicated Carotid Endarterectomy: Tract-based Spatial Statistics Analysis

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**BACKGROUND:** Carotid endarterectomy (CEA) might improve cognitive function. Fractional anisotropy (FA) values in the cerebral white matter derived from diffusion tensor magnetic resonance imaging (DTI) correlate with cognitive function in patients with various central nervous system diseases.

**OBJECTIVE:** To use tract-based spatial statistics to determine whether postoperative changes of FA values in the cerebral white matter derived from DTI are associated with cognitive improvement after uncomplicated CEA.

**METHODS:** In 80 patients undergoing CEA for ipsilateral internal carotid artery stenosis (≥70%), FA values in the cerebral white matter were derived from DTI before and 1 month after surgery and were analyzed by using tract-based spatial statistics. Neuropsychological testing, consisting of the Wechsler Adult Intelligence Scale Revised, the Wechsler Memory Scale and the Rey-Osterreith Complex Figure test, was also performed preoperatively and after the first postoperative month.

**RESULTS:** Based on the neuropsychological assessments, 11 (14%) patients were defined as having postoperatively improved cognition. The difference between the 2 mean FA values (postoperative values minus preoperative values) in the cerebral hemisphere ipsilateral to surgery was significantly associated with postoperative cognitive improvement (95% confidence intervals, 2.632-9.877; $P = .008$). White matter FA values in patients with postoperative cognitive improvement were significantly increased after surgery in the whole ipsilateral cerebral hemisphere, in the contralateral anterior cerebral artery territory, and in the watershed zone between the contralateral anterior and middle cerebral arteries.

**CONCLUSION:** Postoperative increase in cerebral white matter FA on DTI is associated with cognitive improvement after uncomplicated CEA.

**KEY WORDS:** Carotid endarterectomy, Cognition, Diffusion tensor magnetic resonance imaging, Fractional anisotropy, White matter

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**ABBREVIATIONS: CEA,** carotid endarterectomy; **DTI,** diffusion tensor magnetic resonance imaging; **FA,** fractional anisotropy; **FLAIR,** fluid-attenuated inversion recovery; **ICA,** internal carotid artery; **IQ,** intelligence quotient; **ROI,** region-of-interest; **SD,** standard deviation; **TBSS,** tract-based spatial statistics; **WAIS-R,** Wechsler Adult Intelligence Scale Revised

Carotid endarterectomy (CEA) can reduce the risk of stroke in appropriately selected patients[1] and might improve cognitive function.[2-6] A recent study reported that 11% of patients undergoing CEA experienced improvements in cognitive function after surgery.[7] In such patients, hemodynamics in the whole cerebral hemisphere ipsilateral to surgery improved

postoperatively.[8] Metabolism and microscopic anatomic architecture in the cerebral cortex and the cerebral white matter may also change along with these postoperative improvements in cognitive function, but the relationship among these factors remains unclear.

Recently, diffusion tensor magnetic resonance imaging (DTI) has been used to quantitatively evaluate alterations in the white matter in patients with various conditions, including mild traumatic brain injury, brain tumors, idiopathic normal pressure hydrocephalus, and Alzheimer's disease.[9-12] Among the parameters derived from DTI, diffusion anisotropy is most commonly quantified by using fractional anisotropy (FA) values.[13] FA values correlate with cognitive function in patients with cerebral small vessel disease, chronic traumatic brain injury, and Alzheimer disease.[14-17]

There are various methods of analyzing FA data, such as region-of-interest analysis, tract-specific analysis, and voxelwise statistical analysis. Although region-of-interest analysis and tract-specific analysis are simple methods that can be used to directly derive values for structures that are anatomically the same among individuals, these methods are limited in that they are sensitive only to changes in those few parts of the brain that they can accurately measure.[11] In contrast, voxelwise statistical analysis does not require prespecification or prelocalization of regions of interest, and it can be used to automatically perform statistical analysis of the whole brain by voxelwise comparisons between 2 groups.[11] Tract-based spatial statistics (TBSS) is a recently developed voxelwise statistical method that has a specific registration algorithm for FA maps and can thereby minimize misregistration.[18] Further, whole-brain analysis using TBSS may be useful for the characterization of white matter changes that occur in patients undergoing CEA, because the hemodynamics in the whole cerebral hemisphere ipsilateral to surgery often changes postoperatively in such patients.[8]

The purpose of the present study was to use TBSS to determine whether postoperative changes of FA values in the cerebral white matter derived from DTI are associated with cognitive improvement after uncomplicated CEA.

## PATIENTS AND METHODS

### Patient Selection

Patients were prospectively enrolled in this study if they were intended to undergo CEA and if they satisfied the following inclusion criteria: age ≤75 years; symptomatic or asymptomatic ipsilateral cervical internal carotid artery (ICA) stenosis (≥70%) on angiography study via magnetic resonance (MR), computed tomography, or arterial catheterization according to the method of the North American Symptomatic Carotid Endarterectomy Trial[1,19]; preoperative useful residual function (modified Rankin disability scale 0 or 1); no infarction in the cerebral cortical area perfused by one or more branches of the middle cerebral artery as confirmed by MR imaging including T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences that was performed 2 weeks before surgery; and provision of written informed consent. Patients with the following postoperative criteria were excluded from the study: presence of episodes of ipsilateral carotid territory ischemic symptoms that had occurred ≤2 months before presentation to our department, new neurological deficits lasting for 2 weeks after surgery, additional ischemic lesions on MR imaging including T2-weighted and FLAIR sequences performed 2 weeks after surgery in comparison with preoperative MR imaging, and cerebral hyperperfusion determined on intraoperative transcranial regional cerebral oxygen saturation monitoring as described below. A 1.5-T whole body imaging system (Signa MR/I; General Electric, Milwaukee, Wisconsin) was used for the evaluation of ischemic lesions before and after surgery. Whether the anterior cerebral artery (ACA) contralateral to surgery was perfused by the ipsilateral ICA (the A1 of the ipsilateral ACA was not hypoplastic and the contralateral A1 was hypoplastic) was also confirmed on postoperative MR angiography by using this 1.5-T imaging system.

This protocol was reviewed and approved by the institutional ethics committee, and written informed consent was obtained from all patients or their next of kin.

### Preoperative and Intraoperative Management

All patients received antiplatelet therapy until the morning of the day on which CEA was performed. Surgery was conducted under general anesthesia. No intraluminal shunt or patch graft was used in these procedures. A bolus of heparin (5000 international units) was given before ICA clamping. A near-infrared spectroscope, TOS 96 (Tostec, Tokyo, Japan) with a dual-channel system was used to continuously measure regional cerebral oxygen saturation throughout surgery, and the presence or absence of the development of cerebral hyperperfusion was determined intraoperatively as previously described.[20]

### FA Measurements by DTI and TBSS Analysis

DTI was performed by using a 3.0-T superconductive MR imager with a gradient slew rate of 150 mT $m^{-1} \cdot ms^{-1}$ (Signa EXCITE HD; GE Healthcare) and an 8-channel head coil within 7 days before and 1 month after surgery. The following pulse sequences were used for DTI covering the entire brain: axial single-shot, spin-echo, echo-planar imaging; repetition time, 10 000 ms; echo time, 66 ms; 6 motion-probing gradient directions (b value 1000 $s/m^2$); matrix size, 128 × 128; field of view, 24 cm; slice thickness, 4.0 mm with 1.5-mm interslice gaps (voxel size, 1.9 × 1.9 × 4.0 mm); number of slices, 24; number of excitations, 3; parallel imaging reduction factor, 2; and acquisition time, 3 minutes 40 seconds.

TBSS analyses for DTI were performed by using FSL 5.0 (FMRIB Software Tools, Oxford, http://www.fmrib.ox.ac.uk/fsl).[18] After skull stripping and correction for spatial misregistration due to eddy currents and head motions, FA values were calculated for whole brain volumes. The FA maps of patients who underwent CEA on the left side were flipped horizontally so that the right and left cerebral hemispheres were defined as the cerebral hemisphere ipsilateral to surgery and the contralateral cerebral hemisphere, respectively. The FA maps were then aligned to the FMRIB58-FA standard space by using a nonlinear registration algorithm. With the use of TBSS implemented in FSL, mean FA images of each subject were created and were then thinned and thresholded at FA >0.20 to generate a mean FA skeleton that represents the centers of major white matter tracts common to all subjects. The FA map of each subject was projected onto the FA skeleton to obtain skeleton FA maps, which were fed into voxelwise analyses to compare the preoperative and postoperative FA values. Further, right hemispheric (ipsilateral to surgery) and left hemispheric (contralateral to surgery) white matter regions-of-interest (ROIs) were defined as an intersection

between the FA skeleton maps and cerebral white matter labels based on the Harvard-Oxford Subcortical Structural Atlas implemented in FSL, as illustrated in Figure 1. Mean FA values in the ipsilateral and contralateral white matter ROIs were then calculated before and after surgery in each subject. Differences between the 2 mean FA values (postoperative values minus preoperative values) were also calculated and defined as the Δ mean FA in each subject.

To determine the location of voxels with postoperative increase in FA value in each cerebral white matter in a subgroup, a voxelwise group comparison of the FA values before and after surgery was performed by using the FSL function "randomize" with 5000 permutations. Then, statistical maps of $P < .05$ for postoperative increase in FA value were obtained by using a paired $t$ test with a correction for multiple comparison by the family-wise error rate.[21]

Ten healthy volunteers (8 men, 2 women; mean age, 33 years; range, 21-55 years) without any history of hypertension, diabetes mellitus, atrial fibrillation, pulmonary disease, or presence of organic brain lesions, including leukoaraiosis or asymptomatic lacunar infarction on MR imaging served as controls. These participants underwent 2 separate DTI studies in the same manner as described above. The interval between the 2 studies ranged from 1 month to 2 months.

### Neuropsychological Evaluation

For each patient, a battery of neuropsychological tests was administered, consisting of the Wechsler Adult Intelligence Scale Revised (WAIS-R),[22] the Wechsler Memory Scale,[23] and Rey-Osterreith Complex Figure test (Rey test).[24] WAIS-R generates a verbal and performance intelligence quotient (IQ). The Rey test evaluates copy and recall of a complex figure. Thus, 5 scores (WAIS-R verbal IQ, WAIS-R performance IQ, Wechsler Memory Scale, Rey copy, and Rey recall) were used to evaluate cognitive function.

The neuropsychological tests were performed within 7 days before surgery and were repeated 1 month after surgery. All examinations were administered by a trained neuropsychologist who was blinded to the patient's clinical information.

Postoperative cognition was categorized as improved or not improved for each patient based on the definition described previously.[7] In brief, 40 healthy volunteers served as controls and underwent the same neuropsychological tests on 2 separate occasions (intertest interval, 1-2 months).[7] Differences in each neuropsychological test score between the 2 tests (the second test score—the first test score) were calculated. For each neuropsychological test score of patients undergoing CEA, a significant increment was defined as a postoperative test score > preoperative score plus the value of the mean + 2 standard deviations (2SD) of the difference between the 2 test scores in the controls. A patient was defined as having postoperative cognitive improvement when there was a significant increment in postoperative neuropsychological scores on one or more of the 5 tests (WAIS-R verbal IQ, WAIS-R performance IQ, Wechsler Memory Scale, Rey copy, and Rey recall).[7]

### Statistical Analysis

Data are expressed as the mean ± SD. Changes between mean FA values of white matter ROIs in each cerebral hemisphere in the first and second studies were evaluated with the use of the Wilcoxon signed rank test. The relationship between each characteristic and Δ mean FA in each cerebral hemisphere was evaluated by using the Mann-Whitney $U$ test. The relationship between each characteristic and postoperative cognitive improvement was evaluated with univariate analysis by the use of the Mann-Whitney $U$ test or the $\chi^2$ test. A multivariate statistical analysis of factors related to postoperatively improved cognition was also performed with the use of a logistic regression model, and odds ratios with 95% confidence intervals were calculated. Variables with $P < .2$ in the univariate analyses were selected for analysis in the final model. For all statistical analyses, significance was set at the $P < .05$ level.

## RESULTS

Over a period of 31 months, a total of 119 patients underwent CEA. Of these, 91 patients satisfied the preoperative inclusion criteria. However, 1 patient experienced a new major neurological deficit that lasted for 2 weeks after surgery, and another patient developed additional asymptomatic ischemic lesions on MR imaging including T2-weighted and FLAIR sequences performed 1 month after surgery in comparison with preoperative MR imaging. Nine additional patients were determined to have cerebral hyperperfusion on intraoperative monitoring of the regional cerebral oxygen saturation with the use of the near-infrared spectroscope. These 11 patients did not undergo DTI study and neuropsychological testing after surgery and were

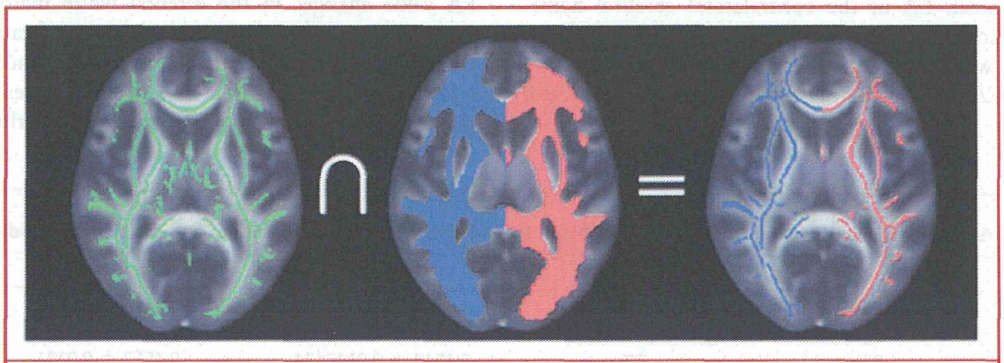

**FIGURE 1.** *Skeleton-based region-of-interest (ROI) analysis procedure. The template for white matter labels in the right (blue) and left (red) cerebral hemispheres (Middle) is registered to the skeleton of the mean fractional anisotropy (FA) map in each subject (green) (Left), resulting in defining ROIs of white matter FA in each cerebral hemisphere (Right).*

excluded from the present study. Thus, the remaining 80 patients were analyzed. None of these patients experienced further ischemic symptoms during the period between initial evaluation and surgical intervention.

The mean age of the 80 patients (73 men, 7 women) was 68 ± 6 years (range, 47-75 years). Concomitant disease states and symptoms were recorded, including 71 patients with hypertension, 32 patients with diabetes mellitus, and 41 patients with dyslipidemia. Fifty patients had ipsilateral symptomatic ICA stenosis, and the remaining 30 patients had asymptomatic ICA stenosis. Preoperative MR imaging demonstrated infarction in the hemisphere ipsilateral to the ICA stenosis in 44 patients and no infarction in 36 patients. Overall average degree of ICA stenosis was 87.2 ± 8.7% with a range of 70% to 99% according to the method of the North American Symptomatic Carotid Endarterectomy Trial.[19] The contralateral ICA was occluded in 3 patients, and 6 additional patients had 60% to 99% stenosis. The ACA contralateral to surgery was perfused by the ipsilateral ICA on MR angiography in 27 patients. Mean duration of ICA clamping was 37 minutes (range, 26-49 minutes). The interval of the neuropsychological testing and measurement of FA was ≤4 days in all patients.

The mean ± SD of mean FA values of white matter ROIs in each cerebral hemisphere in the first and second studies among 80 patients and 10 controls is summarized in Table 1. When analyzed as a group, mean FA values in the 20 cerebral hemispheres in the control participants did not differ between the first study and the second study. In contrast, mean FA values in the cerebral hemispheres ipsilateral and contralateral to surgery in the patients undergoing CEA were significantly greater after surgery than before surgery.

The Δ mean FA in patients ranged from −0.0200 to 0.0392 (0.0037 ± 0.0087) in the cerebral hemisphere ipsilateral to surgery and from −0.0187 to 0.0223 (0.0027 ± 0.0069) in the contralateral cerebral hemisphere. The relationship between each characteristic and Δ mean FA in each cerebral hemisphere is summarized in Table 2. Although variables were not related to Δ mean FA in the ipsilateral cerebral hemisphere, Δ mean FA in the contralateral cerebral hemisphere was significantly greater in patients with bilateral lesions or in patients in whom the contralateral ACA was perfused by the ipsilateral ICA than in patients without those respective features.

Based on the neuropsychological assessments performed before and after surgery, 11 (14%) patients were defined as having postoperatively improved cognition. Results of the univariate analysis of factors related to postoperative cognitive improvement are summarized in Table 3. Patients with postoperative cognitive improvement were significantly younger than those without; the incidence of the bilateral lesions was significantly greater in patients with postoperative cognitive improvement than in those without; the Δ mean FA value in the cerebral hemisphere ipsilateral and contralateral to surgery was significantly greater in patients with postoperative cognitive improvement than in those without. Other variables were not significantly associated with postoperative cognitive improvement. After eliminating variables that were closely related to others, the following items with values of $P < .2$ in univariate analyses were adopted as confounders in the logistic regression model for multivariate analysis: age, bilateral lesions, contralateral ACA perfused by the ipsilateral ICA, and Δ mean FA value in the cerebral hemisphere ipsilateral and contralateral to surgery. This analysis revealed that only Δ mean FA value in the cerebral hemisphere ipsilateral to surgery was significantly associated with postoperative cognitive improvement (95% confidence interval, 2.632-9.877; $P = .008$).

The voxelwise group analysis revealed that white matter FA values in a subgroup of 11 patients with postoperative cognitive improvement were significantly increased after surgery not in the whole ipsilateral cerebral hemisphere, in the contralateral ACA territory, and in the watershed zone between the contralateral ACAs and middle cerebral arteries (Figure 2).

## DISCUSSION

The present study using TBSS analysis demonstrated that postoperative increase in cerebral white matter FA on DTI is associated with cognitive improvement after uncomplicated CEA.

Cerebral ischemic events caused by cerebral hemispheric hypoperfusion during ICA clamping or intraoperative emboli from the surgical site may result in cognitive impairment after CEA.[7] Because we intended to determine the association between FA value changes in the cerebral white matter and cognitive improvement only in patients with uncomplicated CEA, patients with new major neurological deficits and/or additional ischemic lesions on MR imaging after surgery were excluded from the present study. Postoperative cerebral hyperperfusion, even when

**TABLE 1. Comparison of Mean FA Values of White Matter ROIs in Each Cerebral Hemisphere in the First and Second Studies[a,b]**

| | | First Study | Second Study | *P* Value |
|---|---|---|---|---|
| Patients (n = 80) | Ipsilateral hemisphere (n = 80) | 0.4191 ± 0.0293 | 0.4228 ± 0.0291 | <.001 |
| | Contralateral hemisphere (n = 80) | 0.4278 ± 0.0253 | 0.4304 ± 0.0259 | .001 |
| Controls (n = 10) | Bilateral hemispheres (n = 20) | 0.4549 ± 0.0142424 | 0.4552 ± 0.0131 | .467 |

[a]FA, fractional anisotropy; ROIs, regions-of-interest.
[b]Values are expressed as mean ± standard deviation.

**TABLE 2. Relationship Between Characteristics and Δ Mean FA in Each Hemisphere[a]**

| | Δ Mean FA in Ipsilateral Hemisphere | | Δ Mean FA in Contralateral Hemisphere | | |
|---|---|---|---|---|---|
| **Variables** | **Mean ± SD** | ***P* Value** | **Mean ± SD** | ***P* Value** | |
| Age, y | ≥68 (n = 52) | 0.0032 ± 0.0069 | .595 | 0.0024 ± 0.0058 | .057 |
| | <68 (n = 28) | 0.0039 ± 0.0113 | | 0.0041 ± 0.0077 | |
| Sex | Male (n = 73) | 0.0026 ± 0.0091 | .083 | 0.0029 ± 0.0067 | .403 |
| | Female (n = 7) | 0.0066 ± 0.0044 | | 0.0014 ± 0.0055 | |
| Hypertension | Yes (n = 71) | 0.0035 ± 0.0085 | .301 | 0.0034 ± 0.0068 | .192 |
| | No (n = 9) | 0.0015 ± 0.0072 | | 0.0013 ± 0.0074 | |
| Diabetes mellitus | Yes (n = 32) | 0.0028 ± 0.0084 | .294 | 0.0018 ± 0.0080 | .381 |
| | No (n = 48) | 0.0043 ± 0.0091 | | 0.0029 ± 0.0073 | |
| Dyslipidemia | Yes (n = 41) | 0.0026 ± 0.0101 | .166 | 0.0027 ± 0.0078 | .791 |
| | No (n = 39) | 0.0041 ± 0.0073 | | 0.0033 ± 0.0045 | |
| Symptomatic lesion | Yes (n = 50) | 0.0032 ± 0.0094 | .187 | 0.0015 ± 0.0071 | .287 |
| | No (n = 30) | 0.0052 ± 0.0069 | | 0.0035 ± 0.0073 | |
| Infarction on preoperative MRI | Yes (n = 44) | 0.0026 ± 0.0103 | .087 | 0.0024 ± 0.0083 | .702 |
| | No (n = 36) | 0.0051 ± 0.0069 | | 0.0030 ± 0.0061 | |
| Degree of ICA stenosis, % | ≥87 (n = 50) | 0.0039 ± 0.0082 | .639 | 0.0031 ± 0.0057 | .473 |
| | <87 (n = 30) | 0.0031 ± 0.0093 | | 0.0024 ± 0.0082 | |
| Bilateral lesions | Yes (n = 9) | 0.0079 ± 0.0119 | .112 | 0.0094 ± 0.0089 | .011 |
| | No (n = 71) | 0.0031 ± 0.0082 | | 0.0015 ± 0.0055 | |
| Contralateral ACA perfused by ipsilateral ICA | Yes (n = 27) | 0.0073 ± 0.0059 | .076 | 0.0064 ± 0.0055 | .027 |
| | No (n = 53) | 0.0018 ± 0.0093 | | 0.0010 ± 0.0068 | |
| Duration of ICA clamping, min | ≥37 (n = 42) | 0.0049 ± 0.0091 | .714 | 0.0033 ± 0.0062 | .472 |
| | <37 (n = 38) | 0.0034 ± 0.0087 | | 0.0024 ± 0.0076 | |

[a]FA, fractional anisotropy; SD, standard deviation; ICA, internal carotid artery; ACA, anterior cerebral artery; MRI, magnetic resonance imaging.

asymptomatic, is another possible mechanism of cognitive impairment after CEA.[7,25] Intraoperative monitoring of the regional cerebral oxygen saturation using near-infrared spectroscopy is a reliable method of identifying patients with hyperperfusion after CEA.[20,26] Thus, patients characterized as having cerebral hyperperfusion by that mode of monitoring were also excluded from the present study.

In the present study, mean FA values in the cerebral white matter in the controls did not differ between the first study and the second study, indicating that the TBSS analysis has good reproducibility for repeat measurement of FA values. This finding also supports the notion that white matter FA values in the cerebral hemispheres ipsilateral and contralateral to surgery usually increase

**TABLE 3. Comparison of Characteristics Between Patients Who Experienced Postoperative Improvement in Cognition and Those That Did Not[a]**

| | Group | | |
|---|---|---|---|
| | **Improved Cognition (n = 11)** | **No Improvement in Cognition (n = 69)** | ***P* Value** |
| Age, y, mean ± SD | 65.5 ± 5.2 | 68.5 ± 6.4 | .032 |
| Male sex | 82% (9/11) | 93% (64/69) | .245 |
| Hypertension | 91% (10/11) | 88% (61/69) | >.999 |
| Diabetes mellitus | 36% (4/11) | 41% (28/69) | >.999 |
| Dyslipidemia | 64% (7/11) | 49% (34/69) | .520 |
| Symptomatic lesion | 55% (6/11) | 64% (44/69) | .739 |
| Infarction on preoperative MRI | 55% (6/11) | 55% (38/69) | >.999 |
| Degree of ICA stenosis, %, mean ± SD | 86.8 ± 10.1 | 87.2 ± 8.5 | .971 |
| Contralateral ACA perfused by ipsilateral ICA | 55% (6/11) | 30% (21/69) | .169 |
| Bilateral lesions | 36% (4/11) | 7% (5/69) | .018 |
| Duration of ICA clamping, min, mean ± SD | 38.6 ± 5.1 | 37.0 ± 5.7 | .405 |
| Δ mean FA in ipsilateral hemisphere, mean ± SD | 0.0153 ± 0.0091 | 0.0019 ± 0.0072 | <.001 |
| Δ mean FA in contralateral hemisphere, mean ± SD | 0.0082 ± 0.0068 | 0.0018 ± 0.0066 | .024 |

[a]FA, fractional anisotropy; SD, standard deviation; ICA, internal carotid artery; ACA, anterior cerebral artery; MRI, magnetic resonance imaging.

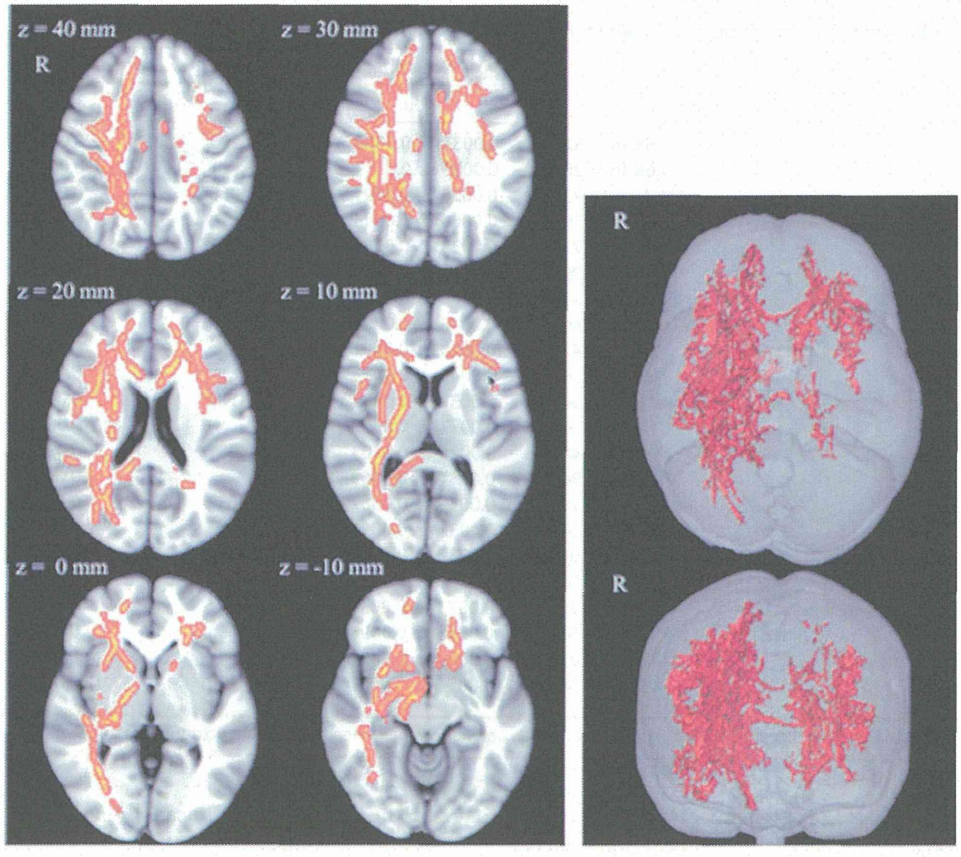


**FIGURE 2.** *Results of voxelwise group analysis for postoperative increase in FA in a subgroup of patients with postoperative cognitive improvement. Voxels with significant postoperative increase in cerebral white matter FA are expressed as red-yellow dots on axial slice images (Left) and axial and coronal 3-dimensional images (Right). Z value in each axial slice image indicates the height from the anterior commissure-posterior commissure line.*

after uncomplicated CEA and that the degree of postoperative increase in white matter FA values in the ipsilateral cerebral hemisphere is significantly associated with postoperative cognitive improvement.

Several studies suggested that diffusion anisotropy is mainly influenced by the magnitude of myelination in animal models[27,28] or remyelination within acute lesions in normal-appearing white matter of patients with multiple sclerosis.[29] A recent study using animal models also demonstrated that remyelination is strongly correlated with the recovery of cognitive dysfunction following chronic cerebral ischemia, suggesting that treatment leading to remyelination could ameliorate the cognitive dysfunction associated with chronic cerebral ischemia.[30] Another study using magnetic resonance spectroscopy showed that choline-containing compounds in the cerebral hemisphere ipsilateral to surgery is increased after CEA in patients with postoperatively improved cognition.[31] This postoperative increase in choline may imply recovery of abnormally reduced myelin membrane turnover (ie, remyelination), resulting in cognitive improvement after CEA.[31] In combination with observations from the present study, these previous findings supported the notion that normalization of cerebral hemodynamics after CEA may lead to remyelination in the cerebral white matter, resulting in postoperative improvements in cognition. However, the correlation between the postoperative increase in brain perfusion and the postoperative increase in FA has not been demonstrated.

In the above-mentioned study that compared postoperative changes in choline (measured by using magnetic resonance spectroscopy) with changes in cognitive function after CEA, magnetic resonance spectroscopy was performed within 7 days before surgery and between 2 and 4 weeks after surgery. Thus, we supposed that the remyelination may occur within 1 month after surgery, and, therefore, DTI was performed at 1 month after surgery in the present study.

In the present study, the presence of bilateral ICA stenoocclusive diseases or the presence of a contralateral ACA perfused

by the ipsilateral ICA was related to the degree of postoperative increase in white matter FA in the cerebral hemisphere contralateral to surgery. Further, the degree of postoperative increase in the white matter FA was greater in patients with postoperatively improved cognition than in those without, although this relationship was not statistically significant on multivariate analysis. Further, according to the voxelwise group analysis, white matter FA values in patients with postoperative cognitive improvement were significantly increased after surgery even in the contralateral ACA territory and the watershed zone between the contralateral ACA and middle cerebral artery. These data suggested that improvement in cerebral hemodynamics in the contralateral cerebral hemisphere after CEA leads to remyelination in the white matter in that cerebral hemisphere, which may, in part, contribute to postoperative cognitive improvement.

## CONCLUSION

The present study using TBSS analysis demonstrated that postoperative increase in cerebral white matter FA on DTI is associated with cognitive improvement after uncomplicated CEA. Normalization of cerebral hemodynamics after CEA may lead to remyelination in the cerebral white matter, resulting in postoperative improvements in cognition.

### Disclosures

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## COMMENTS

The article entitled "Postoperative Increase in Cerebral White Matter Fractional Anisotropy on Diffusion Tensor Magnetic Resonance Imaging Is Associated With Cognitive Improvement After

Uncomplicated Carotid Endarterectomy: Tract-Based Spatial Statistics Analysis" demonstrates an important issue related to cognitive performance, ie, cognitive domains encompass multiple brain regions all of which participate in cognitive performance. The authors have demonstrated that improved cognitive function in white matter connectivity by using new techniques: fractional anisotropy (FA) in the cerebral white matter derived from diffusion tensor magnetic resonance imaging, and a new method of analyzing FA data, tract-based spatial statistics (TBSS). It would be interesting to see if cognitive dysfunction also had its correlates in changes in FA. The present study is one of the first articles to demonstrate the relation of changes of anatomic architecture in the cerebral white matter and improvements in cognitive function after CEA.

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This is a rigorous and extremely well done study that characterizes the previously reported phenomenon of improved cognitive function following carotid endarterectomy and assesses whether this improvement relates to cerebral white matter changes after surgery through use of Fractional anisotropy (FA) magnetic resonance imaging measurements. Their results demonstrate again that in a small but significant number of patients (14% in this series) carotid endarterectomy appears to improve cognition - presumably by addressing a deficit in cerebral hemodynamics. Potential confounds such as inclusion of patients with recent symptomatic stroke (a population where cognitive improvement over time would be expected) were appropriately excluded. They also convincingly showed that the change in FA following surgery correlated with postoperative cognitive improvement.

Because FA improvements have been linked in preclinical and clinical studies to remyelination, the authors conclude that post-endarterectomy improvements in cerebral hemodynamics lead to white matter remyelination and ultimately enhanced cognitive function. This may very well be the case, but it is important to note that cerebral hemodynamics was not assessed in this study. We do not know whether the patients that showed improved cognition following surgery were in fact those that had impaired cerebral hemodynamics pre-operatively. Additional studies will be required to make this causal link. Overall, this is a high quality study that provides support for the notion that cognitive dysfunction should be included in future clinical trials examining indications and benefits of carotid endarterectomy. It also argues that FA might be useful as a surrogate marker for this clinical endpoint.

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# Deficiency of senescence marker protein 30 exacerbates angiotensin II-induced cardiac remodelling

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**Aims** Ageing is an important risk factor of cardiovascular diseases including heart failure. Senescence marker protein 30 (SMP30), which was originally identified as an important ageing marker protein, is assumed to act as a novel anti-ageing factor in various organs. However, the role of SMP30 in the heart has not been previously explored. In this study, our aim was to elucidate the functional role of SMP30 on cardiac remodelling.

**Methods and results** SMP30 knockout (KO) mice and wild-type (WT) mice were subjected to continuous angiotensin II (Ang II) infusion. After 14 days, the extent of cardiac hypertrophy and myocardial fibrosis was significantly higher in SMP30-KO mice than in WT mice. Echocardiography revealed that SMP30-KO mice had more severely depressed systolic and diastolic function with left ventricular dilatation compared with WT mice. Generation of reactive oxygen species related with activation of nicotinamide adenine dinucleotide phosphate-oxidase was greater in SMP30-KO mice than in WT mice. The number of deoxynucleotidyl transferase-mediated dUTP nick end-labelling positive nuclei was markedly increased in SMP30-KO mice with activation of caspase-3, increases in the Bax to Bcl-2 ratio and phosphorylation of c-Jun N-terminal kinase compared with WT mice. Furthermore, the number of senescence-associated β-galactosidase-positive cells was significantly increased via up-regulation of p21 gene expression in SMP30-KO mice compared with WT mice.

**Conclusion** This study demonstrated the first evidence that deficiency of SMP30 exacerbates Ang II-induced cardiac hypertrophy, dysfunction, and remodelling, suggesting that SMP30 has a cardio-protective role in cardiac remodelling with anti-oxidative and anti-apoptotic effects in response to Ang II.

**Keywords** Senescence marker protein 30 (SMP30) • Ageing • Remodelling • Oxidative stress • Apoptosis

## 1. Introduction

The prevalence and mortality rate of heart failure dramatically increase in older people, and ageing is one of the risk factors for cardiovascular events.[1] With ageing, the heart shows changes in cardiac structure and function. Age-associated cardiac remodelling includes an enlargement of cardiomyocyte size, loss of myocytes due to apoptosis or necrosis, and increase of matrix connective tissue. These age-associated cardiac changes seem to be relevant to the steep increases in left ventricular hypertrophy, diastolic dysfunction, and subsequent heart failure.[2]

Oxidative stress is considered to be an important factor in controlling heart ageing.[3] It is well known that the renin–angiotensin system (RAS) is a central component of the physiological and pathological responses of the cardiovascular system. Activation of RAS is a significant driver of oxidative stress and is involved in age-related cardiac remodelling. Angiotensin II (Ang II), the primary effector molecule of RAS, contributes not only to vasoconstriction and hypertension, but also to cardiac hypertrophy, remodelling, and heart failure. Therefore, Ang II signalling appears to play a critical role in heart ageing.

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Senescence marker protein 30 (SMP30), a 34-kDa protein, was originally identified as a novel ageing marker protein in rat liver, whose expression decreases androgen-independently with age.[4] SMP30 transcripts are detected in almost all organs, and the SMP30 gene is highly conserved among numerous animal species including humans.[5] Intracellular localization of SMP30 is in the cytoplasm and perinuclear regions, and SMP30 exists in multiple forms under physiological conditions.[6] It has been demonstrated that SMP30 plays multifunctional roles as $Ca^{2+}$ regulator (named as regucalcin),[7] anti-oxidant,[8] and enzymatic ability to hydrolyze di-isopropyl phosphorofluoridate.[9] Recently, SMP30 has been determined as gluconolactonase, which is involved in ascorbic acid (vitamin C) biosynthesis in mammals, whereas human beings are unable to synthesize vitamin C *in vivo* because of mutations in L-gulonolactone oxidase.[10]

SMP30-knockout (KO) mice have been generated[11] and showed a shorter life span than that of the wild-type (WT) mice on a vitamin C-deficient diet.[12] Using SMP30-KO mice, recent reports have demonstrated that SMP30 functions to protect cells from apoptosis in the liver[11] and that SMP30 has protective effects against age-associated oxidative stress in the brain[13] and lungs.[14] Furthermore, SMP30-KO mice have shown accelerated senescence in the kidney[15] and the worsening of glucose tolerance.[16] Taken together, SMP30 is assumed to behave as an anti-ageing factor. However, the role of SMP30 in the heart has not been previously explored.

We hypothesized that SMP30 has cardio-protective functions from harmful stimuli with anti-oxidative and anti-apoptotic effects. To test the hypothesis, we used SMP30-KO mice to examine the effects of SMP30 on Ang II-induced cardiac hypertrophy and remodelling *in vivo*.

# 2. Methods

For additional detailed methods, please see Supplementary material online..

## 2.1 Animal protocol

SMP30-KO (C57BL/6 background) mice were established as previously reported.[11] Drinking water containing vitamin C (1.5 g/L) was provided for the SMP30-KO mice to avoid vitamin C deficiency due to their inability to synthesize vitamin C *in vivo*.[10] After anaesthetizing the mice by i.p. injection of pentobarbital (50 mg/kg body weight), an osmotic minipump (ALZET micro-osmotic pump MODEL 1002, DURECT Co., Cupertino, CA, USA) was subcutaneously implanted, and Ang II (800 ng/kg/min) was continuously infused for 14 days.[17,18] Controls were administered saline. The investigations conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication, 8th Edition, 2011). Our research protocol was approved by the institutional review board, and all animal experiments were conducted in accordance with the guidelines of Fukushima Medical University Animal Research Committee.

## 2.2 Measurement of vitamin C

Total vitamin C levels in the heart were measured by the dinitrophenylhydrazine method according to the manufacturer's protocol (SHIMA Laboratories Co. Ltd., Tokyo, Japan).[19]

## 2.3 Measurements of blood pressure and heart rate

Mice were implanted with a radiotelemetry probe (TA11PA-C22, Data Sciences International, St Paul, MN, USA) under i.p. anaesthesia by pentobarbital (50 mg/kg body weight) as described previously.[20] After a recovery phase of 10 days, basal arterial pressure and heart rate (HR) were started to recorded. After the measurement of control, Ang II was subcutaneously infused via an osmotic minipump, and the data were recorded.

## 2.4 Echocardiography

Transthoracic echocardiography was performed using Vevo 2100 High-Resolution *In Vivo* Imaging System (Visual Sonics, Inc., Toronto, Canada) with a high-resolution 40-MHz imaging transducer as previous reports described.[21,22] Mice were lightly anaesthetized by titrating isoflurane (0.5–1.5%) to achieve an HR of ~400 b.p.m., and all the measurements were obtained from three cardiac cycles.

## 2.5 Cardiac catheterization

The cardiac catheterization was performed as described previously.[23] Briefly, mice were anaesthetized by i.p. injection of 2,2,2-tribromo-ethanol (250 mg/kg body weight), the right carotid artery was cannulated with the micropressure transducer (samba preclin 420 LP, Samba Sensors AB, Gothenburg, Sweden) into the left ventricle. Adequacy of anaesthesia was monitored by HR, aortic blood pressure, and respiratory rate as well as the absence of reactions of painful stimuli. The data were measured using the Labscribe 2 software (iWorx Systems, Inc., Dover, NH, USA).

## 2.6 Histopathological analysis

After continuous infusion of Ang II or saline for 14 days, mice were sacrificed by cervical dislocation and hearts were rapidly excised. The paraffin-embedded heart sections were stained with haematoxylin and eosin or Elastica-Masson. The cross-sectional area of cardiomyocyte and fibrosis fraction was measured using the NIH ImageJ software (National Institutes of Health, Bethesda, MD, USA) and Adobe Photoshop CS2 (Adobe, San Jose, CA, USA).[24]

In immunohistochemical analysis, the paraffin-embedded sections were incubated with anti-SMP30 antibody (SHIMA Laboratories Co. Ltd., Tokyo, Japan) with a dilution of 1:200 or negative control (normal serum). The sections were stained with horseradish peroxidase-conjugated secondary antibody (Histofine Simple Stain Mouse MAX PO (R), Nichirei Biosciences, Inc., Tokyo, Japan) and diaminobenzidine etrahydrochloride, and counterstained with haematoxylin.

## 2.7 Assessment of reactive oxygen species generation

The fresh-frozen heart sections were incubated with 10 μmol/L dihydroethidium (DHE, Sigma-Aldrich Co., St Louis, MO, USA).[25,26] The fluorescent images were acquired using fluorescence microscope (Olympus IX71, OLYMPUS Optical Co., Tokyo, Japan) and the mean DHE fluorescence intensity of cardiomyocytes was quantitated with the NIH imageJ software.[26] In addition, 10 mmol/L apocynin, a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor, was provided in drinking water with Ang II continuous infusion, and reactive oxygen species (ROS) generation was evaluated by DHE staining.[27]

## 2.8 Western blotting

Total protein was extracted from the snap-frozen left ventricle using Cell Lysis Buffer (Cell Signaling Technology, Inc., Beverly, MA, USA) with Protease Inhibitor Cocktail (BD Biosciences, San Jose, CA, USA) as previous reports described.[24] The primary antibodies were as follows: anti-SMP30, anti-$67^{phox}$, anti-Bax, anti-Bcl-2, anti-phospho-stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK, Thr183/Tyr185), anti-SAPK/JNK (Cell Signaling Technology, Inc.), anti-activated-caspase-3 (Bioworld Technology, Inc., Minneapolis, MN, USA), and mouse anti-β-actin (Santa Cruz Biotechnology, Inc.). The signals from immunoreactive bands were visualized by an Amersham ECL system (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, UK) and quantified using densitometric analysis.