Impairments in Cognitive Function and Brain Connectivity in Severe Asymptomatic Carotid Stenosis

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Background and Purpose—Severe asymptomatic carotid stenosis has been associated with cognitive impairment, but it is unknown whether this association is attributable to effects on brain connectivity. We present cognitive network abnormalities in a group of patients at a presymptomatic stage.

Methods—Seventeen patients with ≥70% asymptomatic stenosis of unilateral internal carotid artery were compared with 26 healthy controls utilizing a comprehensive neuropsychological battery, the dizziness handicap inventory, and multimodality neuroimaging including diffusion tensor imaging and resting-state functional connectivity magnetic resonance imaging. Longitudinally, assessments were completed in a subgroup of 10 patients at 3 months after carotid artery stenting.

Results—Compared with the healthy controls, the patients had worse dizziness scores, poorer memory, complex visuo-spatial performances, and lower whole-brain mean fractional anisotropy. The Scheltens scores of leukoaraiosis/infarction were not different between groups. Their seed-based functional connectivity magnetic resonance imaging showed marked decrements of interhemispheric and intrahemispheric, ipsilaterally to carotid stenosis, functional connectivity in the frontoparietal network. In the default mode network, the intrahemispheric functional connectivity was bilaterally impaired. Importantly, the disrupted mean fractional anisotropy in the patients significantly correlated with the attention and verbal memory functions. After successful carotid artery stenting, small but measurable increments of the mean fractional anisotropy and little functional connectivity in the default mode network ipsilateral-to-carotid artery stenting were noted.

Conclusions—We identified for the first time distinct patterns of network disruption that correlate with cognitive fragility in patients with asymptomatic carotid stenosis. Brain connectivity may provide early and useful biomarkers for brain ischemia and reperfusion. (Stroke. 2012;43:2567-2573.)

Key Words: asymptomatic carotid stenosis ■ cognitive impairment ■ diffusion tensor imaging ■ endovascular treatment ■ functional connectivity magnetic resonance imaging ■ resting state ■ stent

Asymptomatic stenosis of the internal carotid artery (ICA) is described as significant atherosclerosis without stroke or transient ischemic attack in the brain or eyes.1 Retrospective studies comparing asymptomatic subjects with and without ultrasound-assessed carotid stenosis have shown that subjects with carotid stenosis had significantly poorer performance in tests of attention, psychomotor speed, and memory.2-4 Moreover, severe ICA stenosis (≥50%) has been associated with a higher prevalence of silent cerebral infarcts and white matter hyperintensities,5 indicating that “asymptomatic” carotid stenosis may not be truly asymptomatic. To understand the pathological changes after chronic cerebral hypoperfusion, experimental models of vascular cognitive impairment have been induced by bilateral5 or unilateral6 common carotid artery occlusion. Mice subjected to unilateral carotid artery occlusion had development of impaired object recognition and significant white matter damage in the corpus callosum and frontal-subcortical circuits; however, they maintained normal spontaneous activity.6 Once their cerebral ischemia reached a severe degree (<10% supply), it caused a rapid loss of spine and dendrite microstructure within 10 minutes, which could be partially reversible only when reperfusion occurred within 20 to 60 minutes.7 In the clinical scenario of carotid revascularization, a few studies showed that patients with severe asymptomatic ICA stenosis had cognitive improvements 3 months after carotid artery stenting.
CAS), particularly in psychomotor processing speed; however, this conclusion is being debated because of lack of controls. It is unknown whether and how their cognitive alterations are related to disruptions in brain connectivity.

Diffusion tensor imaging (DTI) and resting-state functional connectivity magnetic resonance imaging (fcMRI) have been increasingly used to analyze brain connectivity (ie, structural and functional connectivity, respectively) in neuropsychiatric diseases. DTI measures the restricted diffusivity of water molecules, known as fractional anisotropy (FA), in a spatially coherent manner and characterizes white matter tracts. In fcMRI, the spatio-temporally organized coherence of spontaneous blood oxygenation level-dependent signals at low frequencies (<0.1 Hz) is thought to reflect the intrinsic networks of the brain. The significant correlations between brain regions, the so-called functional connectivity (FC), has been used to identify resting-state networks, including the sensorimotor network, the default mode network, the so-called functional connectivity (FC), and the dorsal attention network. Disrupted FC in the DMN, particularly disruption in the posterior cingulate cortex (PCC) and in the hippocampus, has been implicated in mild cognitive impairment and Alzheimer disease. Nevertheless, it has not been studied whether complementary DTI and fcMRI may detect microstructural and functional alteration of neural networks in severe asymptomatic carotid stenosis and after carotid revascularization.

In this work, we investigated neuropsychological performance, DTI, and fcMRI in 17 consecutive patients with severe asymptomatic carotid stenosis and compared them with 26 healthy subjects. To our knowledge, this is the first study to demonstrate the disconnections of structural and functional connectivity in correlation with cognitive impairments in patients with severe asymptomatic carotid stenosis.

**Subjects and Methods**

**Subjects and Neuropsychological Tests**

We recruited consecutive, testable patients with severe, unilateral stenosis (>70% by both ultrasound and magnetic resonance angiography; contralateral carotid stenosis <50% if any) of the extracranial ICA from our dizziness/cerebrovascular clinic during the period between February 2010 and January 2012. Many of them had symptoms of dizziness and amnesia. We also recruited age-eligible, and education level–eligible healthy controls without significant (>50%) stenosis of large cerebral arteries. All of the subjects were 55 to 80 years of age, right-hand-dominant, and free of stroke, transient ischemic attack, dementia, and depression. Other exclusion criteria included the presence of any comorbid neurological disease (such as Parkinson disease), severe medical diseases (such as advanced malignancy, heart failure, sepsis, cirrhosis, renal failure, and chronic obstructive pulmonary disease), or functional disability (modified Rankin Scale ≥3). A blinded rater evaluated the participants using the dizziness handicap inventory and a battery of neuropsychological tests, including the Mini-Mental State Examination, the Taiwan Geriatric Depression Scale, a working memory test (backward digit span), a verbal memory test (immediate recall and delayed recall), an attention test (the symbol digit test, as a part of the Wechsler Adult Intelligence Scale), executive function tests (the modified trail-making test A and B, trail-making test A/B, and the Stroop color-word test), and a complex visuospatial perception test (the modified complex figure test with copy and recall). The Ethics Committee of the Taipei Veterans General Hospital (VGHIRB 98-08-4A, 2011-01-0071C, 2011-12-009GA) approved this study. Each subject signed the consent form in advance.

**MRI Acquisition**

The images were acquired with a 3.0 GE Discovery 750 MRI scanner. A standard head coil with foam padding was used to restrict head motion. All of the imaging sections were acquired along the anterior–posterior commissural plane, as identified by multiplanar T1-weighted BRAVO anatomic images (repetition time, 12.2 ms; echo time, 5.2 ms; flip angle, 12 degrees; voxel size, 1×1×1 mm; field of view, field of view = 256 mm). Standard fluid attenuation inversion recovery images were also acquired to assess structural lesions. For the fcMRI, we recorded blood oxygenation level-dependent signals from 1 task-free run (124 time points/372 s) with a gradient-echo echo-planar imaging sequence (repetition time/echo time, 3000/30 ms; flip angle, 90 degrees; field of vision, 222 mm; thickness, 3 mm). The subjects were asked to open their eyes without thinking or moving. For the DTI, we used a single-shot diffusion spin-echo echo-planar imaging sequence (repetition time/echo time, 9500/85.6 ms; thickness, 2 mm; matrix, 128×128; field of vision, 256 mm; 30 directions). The MRI was longitudinally acquired twice in patients receiving CAS (before CAS and 3 months after the CAS).

**MRI Analysis**

A blinded neurologist assessed all of the images. The presence of lacunes (diameter <15 mm) and leukoaraiosis (hypointensity on the T1-weighted images and hyperintensity on the fluid attenuation inversion recovery images) were all depicted on a Montreal Neurological Institute template with the MRcron software. The semi-quantitative Scheltens scale for leukoaraiosis/infarcts is evaluated in details in the Supplementary Methods.

For the DTI analysis, the Diffusion Toolbox package and tract-based spatial statistics from the FMRIB Software Library (FSL 3.2, http://www.fmrib.ox.ac.uk/fsl) were used to perform preprocessing and voxel-wise FA analysis, respectively. The details are described in the Supplementary Methods. For the FA maps and FC correlation maps, the hemisphere ipsilateral to the carotid stenosis was set to the left side by flipping along the mid sagittal axis.

For the fcMRI analysis, a preprocessing procedure was performed using our previously described method (details provided in the Supplementary Methods). Regions of interest (ROI) representing the seed regions for 3 resting-state networks, including the DMN, the FPN, and the dorsal attention network, were predefined according to the literature (Supplementary Table I). All of the ROI were 4 mm in radius. The Pearson correlation coefficient (r) for the temporal correlation between the blood oxygenation level–dependent signals from each ROI-to-ROI pair, ie, the FC value, was calculated with a Fisher r-to-z transformation to yield an approximately normally distributed measurement. The voxel-wise correlation z maps from each single ROI were computed with 1-sample t test by using The Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology in London) for within-group analysis. We then compared the mean (homologous) interhemispheric and intrahemispheric FC values of the ROI-to-ROI pairs between the 2 groups (a total of 17 ROI pairs compared: 9 in DMN; 4 in FPN; 4 in dorsal attention network) and between the baseline and the 3-month follow-up periods in the patients receiving CAS.

**Statistical Analysis**

We used the SPSS software (version 18.0) for data analyses. The χ² or Fisher exact test (when the expected number ≤5) was used to compare the categorical demographic variables. The nonparametric Mann-Whitney U test was used to compare the dizziness scores, the neuropsychological scores (Bonferroni correction n = 10 items), the Scheltens scores, the FC, and the mean FA values between groups. To investigate these longitudinal changes after CAS, we adopted the nonparametric Wilcoxon signed-rank test for differences between the baseline and the 3-month follow-up. Significance was defined as a corrected P<0.05.
Results

Patient Characteristics and Neuropsychological Evaluation

We enrolled 17 consecutive patients with unilateral, severe, asymptomatic carotid stenosis and 26 healthy controls (Table 1). There were no significant differences in age, gender ratio, educational years, depression scores, and vascular risk factors between groups. Compared with the controls, the patients had significantly worse dizziness scores and poorer cognitive performance on the memory (working and verbal) and complex visuospatial perception tests. The Mini-Mental State Examination scores and the attention and the executive scores were not different after corrections for multiple comparisons (Table 2).

White Matter Hyperintensities and Impaired Structural Connectivity

The semi-quantitative Scheltens scores were not different between groups (6.6±3.6 vs 5.6±2.4; P=0.44; Figure 1A). Nevertheless, the patient group had lower whole-brain mean FA (0.45±0.03 vs 0.6±0.02; P<0.001) and diffuse decrements of FA compared with the control group (Figure 1B, C) in bilateral white matter tracts, particularly in frontoparietal regions ipsilateral to the stenosis and in the PCC. These findings are indicative of poorer diffusivity and microstructural disruption of white matter integrity.

Brain Connectivity in Correlation With Neuropsychological Measurements

Compared with the healthy controls, the patients had a markedly decreased blood oxygenation level–dependent correlation in the opposite hemisphere when the ROI were predefined on the stenotic side (flipped to the left) (Figure 2A), suggesting a disruption of interhemispheric connectivity. The within-group analysis performed with 1-sample t test also showed that the patients had a more asymmetrical network distribution in the cortical surface areas (Figure 2B). Especially in the FPN, the long-distance interhemispheric FC between the bilateral dorsal lateral prefrontal cortices (q=0.033) and between the bilateral anterior inferior parietal lobules (q=0.007) were significantly reduced in the patients compared with the controls (Figure 2C). The short-distance interhemispheric FC between the bilateral hippocampi in the DMN was preserved. Also, the intrahemispheric FC was specifically decreased between the dorsal lateral prefrontal cortices and the anterior inferior parietal lobules in the FPN ipsilateral to the stenosis (q=0.021), and so were the FC between the PCC and the medial prefrontal cortex (q=0.022), and between the PCC and the hippocampus bilaterally (ipsi-
lateral \( q = 0.009 \) and contralateral \( q = 0.014 \), respectively) in the DMN compared to those of the healthy controls (Figure 2D). Taken together, the interhemispheric FC in the FPN and the intrahemispheric FC in the FPN (ipsilateral to the stenosis) and in the DMN were specifically susceptible in the patients with severe asymptomatic carotid stenosis.

The whole-brain mean FA significantly correlated with the symbol digit test scores of attention function (\( P = 0.005 \)) and...
the immediate recall scores of verbal memory function \((P=0.03)\) in the patients, but not in the controls (Figure 3). There were no case-control differences in the symbol digit test or the immediate recall scores (Table 2). In contrast, we did not find correlations between the Scheltens scores and the neuropsychological measures (data not shown). There were no correlations of the mean FA with the dizziness scores, the working memory (backward digit span), or the executive (trail-making test B) scores. Taken together, decreased whole-brain mean FA values in the patients are indicative of attention and verbal memory impairments.

### Carotid Revascularization on Cognitive Functions and Brain Connectivity

We investigated the changes in cognitive function and brain connectivity after CAS at 3 months with an uncontrolled design. Eleven of the 17 patients accepted CAS rather than carotid endarterectomy (CAS is one of the hospital’s best-known procedures), and the other 6 preferred aggressive medical treatments only. Successful carotid revascularization (defined as being free of periprocedural complications within 1 month and having residual stenosis \(<50\%)\) was achieved in all 11 patients (100%), and 10 of them completed the longitudinal follow-up (Supplementary Table II). After CAS, these patients had insignificant improvements in their dizziness and the neuropsychological scores after corrections for multiple comparisons (Figure 4D). Interestingly, small but measurable increases in the mean FA \((P=0.017;\) Figure 4A) and in the FC between the posterior inferior parietal lobules and the hippocampus ipsilateral to CAS \((q=0.035)\) and in that between the PCC and the medial prefrontal cortex \((q=0.027)\) in the DMN were observed (Figure 4B, C), implying a causal relationship between reperfusion and connectivity increments. In addition, we found that 2 of the 10 patients had 1 to 3 asymptomatic new MRI embolic infarctions after CAS (data not shown), which might have had an impact on cognitive functions, although it was not evident in our examinations at 3-month follow-up, consistent with a previous randomized comparison between the CAS and carotid endarterectomy.33

### Discussion

Here, we show that patients with severe asymptomatic ICA stenosis had more dizziness, poorer cognitive performance (including working and verbal memory and complex visuospatial perception). Importantly, the patients had further diffuse impairments of white matter structural connectivity (FA) and regionally specific disruptions of FC in the FPN and the DMN. The disrupted mean FA in the patient group correlated significantly with the attention and the verbal memory impairments. Their cognitive impairments are likely attributed to both structural and functional disconnections. The DTI and fcMRI in these patients provide sensitive and objective information to detect network abnormalities early, even at a presymptomatic stage.

Our major finding is that patients with severe asymptomatic unilateral carotid stenosis had significantly reduced whole-brain mean FA and FC, particularly in the interhemispheric FPN (between bilateral dorsal lateral prefrontal cortices and bilateral anterior inferior parietal lobules) and the intrahemispheric FPN (dorsal lateral prefrontal cortices–anterior inferior parietal lobules ipsilateral to the stenosis) and the DMN (PCC–hippocampus and PCC–medial prefrontal cortex). The PCC, supplied by the precuneal artery from its origins at the ICA, has been shown to be particularly vulnerable and is involved in degenerative cognitive decline.18,19,34 The hippocampus is mainly supplied by the posterior cerebral artery and was less affected in these patients. Moreover, the medial prefrontal cortex and the frontoparietal region receive blood from the ICA, and therefore they are directly affected. Consistent with other findings, the disrupted connectivity between these hubs were correlated with cognitive impairments.18,19 Importantly, significant impairment of FA supports the aforementioned functional discontinuity and pro-

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**Figure 3.** Scatter plots of the correlation analyses between the whole-brain mean fractional anisotropy (FA) and (A) the symbol digit test scores (attention function), (B) the immediate recall scores (verbal memory function), and (C) the dizziness scale scores. Regression lines are calculated separately in the control (HC, dashed lines) and the patient (PA, solid lines) groups. All probability values are adjusted by Bonferroni correction.
Figure 4. The changes in brain connectivity and neuropsychological measurements at 3 months after carotid artery stenting (CAS). A, A modest increase in fractional anisotropy (FA) (red to yellow; P<0.05) and the whole-brain mean FA value (bar chart of baseline [pre] and 3 months [3 m] after CAS). B and C, Note few increments of intrahemispheric FC in the default mode network (DMN) at the medial prefrontal cortex (MPFC) and the hippocampus (HP) ipsilateral to the CAS (arrowheads). *q<0.05, false discovery rate corrected. D, The dizziness scores and the neuropsychological scores were insignificantly changed after the CAS. DHI, dizziness handicap inventory; BDS, backward digit span; SDT, symbol digit test; TMT-A/B: trail-making test A/B. The error bars represent the standard errors of the mean.

Conclusions

Patients with severe asymptomatic carotid stenosis have an increased risk of cognitive fragility and white matter tract disruption. The FPN and DMN have high susceptibility to chronic cerebral hypoperfusion. Complementary DTI and fc-MRI in these patients provide sensitive and objective information on the functional status of the network connectivity.

that is sufficient to produce abrupt cognitive impairment beyond a standard deviation from the mean.37 Given the common cardiovascular risk factors in the elderly with either vascular or nonvascular cognitive impairment18 and the high prevalence of mixed pathologies of microvascular infarct and Alzheimer amyloid plaque,39 early identification of treatable vascular condition is warranted, even in those with neurodegenerative diseases.

The impact of carotid revascularization on cognition and brain connectivity needs further investigation given the limitation of the small subpopulation subjected to CAS. Because of the class II recommendations (benefit greater than risk) for carotid revascularization in selected asymptomatic patients who have ≥70% stenosis of the ICA and low perioperative risk,40 there has been hesitation for randomized controlled studies for the effects of CAS and the uncontrolled observations therefore have been challenged.9,11 We found small but measurable increments of the whole-brain mean FA and focal FC in the DMN ipsilateral to CAS. Nevertheless, the neuropsychological measures and most disrupted FC did not change obviously after statistical adjustments. The strengthened FC neighbor to some disrupted areas at 3 months after CAS may be attributable to restored perfusion and vasomotor reactivity,20 enhanced axonal transport, and/or functional plasticity of already existing pathways. A study with a larger population and longer observation is needed to determine whether cognitive impairment can be enhanced after CAS and whether the increased brain connectivity may correlate with cognitive improvement.

There are several limitations to this study. First, the sample size and the number of multiple comparisons made between patients and controls were small. Second, we used a rapid fcMRI acquisition (≈6 minutes) to estimate the FC strength. A single 4-minute fcMRI run is sufficient to estimate connectivity,26,41 but increasing the acquisition time can improve the signal-to-noise ratio. Third, blood oxygenation level–dependent signals are sensitive to impaired perfusion and vasomotor reactivity in cerebrovascular diseases in which the coupling between neuronal activity and hemodynamic activity has been affected.42 Hence, we adopted complementary DTI and had been cautious in interpretation of these changes. Further investigation using electroencephalography or magnetoencephalography can provide direct evidence for neural activity. Fourth, we compared FC of homologous ROI pairs between groups and might have underestimated differences outside the ROI we selected a priori or between nonhomologous ROI pairs. A brain-wise comparison between groups is currently undergoing investigation. Given the small sample size and the uncontrolled results of the comparisons before and after CAS, larger studies to investigate the alternations found in brain connectivity are needed in the future.

Consistent with previous studies of patients with asymptomatic carotid stenosis,2,4,15 our patients showed poorer cognitive performance than the healthy controls, but not in Mini-Mental State Examination. All of the patients’ Mini-Mental State Examination scores were >24, which is a cut-off value for mild dementia suggested by the large community-based domestic (Taiwan) studies of healthy elderly subjects (26.52±2.93).22,36 Hence, cognitive alterations in asymptomatic patients with severe carotid stenosis may not meet the criteria of vascular cognitive impairment, which has been vaguely constructed to encompass any vascular disorder

that is sufficient to produce abrupt cognitive impairment beyond a standard deviation from the mean.37 Given the common cardiovascular risk factors in the elderly with either vascular or nonvascular cognitive impairment18 and the high prevalence of mixed pathologies of microvascular infarct and Alzheimer amyloid plaque,39 early identification of treatable vascular condition is warranted, even in those with neurodegenerative diseases.

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Acknowledgments
The authors thank Chia-Feng Lu for technical assistance in the image analysis.

Sources of Funding
The National Science Council (NSC-100-2314-B-075-012, NSC-99-3111-B-010-004), the National Yang-Ming University (101AC-B16), and the Taipei Veterans General Hospital (V100C-045, V100E-009, V101C-062, V101E-008) in Taiwan supported this work.

Disclosures
None.

References
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Stroke. 2012;43:2567-2573; originally published online August 30, 2012; doi: 10.1161/STROKEAHA.111.645614

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SUPPLEMENTAL MATERIAL

Impairments in cognitive function and brain connectivity in severe asymptomatic carotid stenosis

Supplemental Methods

MRI Scheltens scale
The whole brain was divided into periventricular, deep white matter and basal ganglia. The periventricular region was further divided into the frontal caps, occipital caps and the bands beside lateral ventricles. Each region was separately scored as follows: absent as 0, white matter lesions up to 5 mm as 1 and larger than 5 mm as 2. The deep white matter was divided into frontal, parietal, occipital and temporal regions. The basal ganglia were divided into caudate nucleus, putamen, globus pallidus, thalamus and internal capsule. Each of these regions was scored as follows: absence of lesions as 0, <6 lesions with a diameter smaller than 3 mm as 1, >5 lesions with a diameter smaller than 3 mm as 2, <6 lesions with a diameter of 4–10 mm as 3, >5 lesions with a diameter of 4–10 mm as 4, at least one lesion 10 mm diameter as 5, and confluent lesions as 6. All of the scores of the different regions were summed to obtain a total scale score of an individual.

DTI analysis
The Diffusion Toolbox package and tract-based spatial statistics (TBSS) from the FMRIB Software Library (FSL 3.2, http://www.fmrib.ox.ac.uk/fsl) were used to perform preprocessing and voxel-wise FA analysis, respectively. The raw DTI images were corrected for eddy currents and head motion. To create a brain mask from the b=0 images, the non-brain structures were removed using the brain extraction tool in FSL. The diffusion tensor was then fitted to a diffusion tensor model at each voxel of the preprocessed data using the DTIfit program in FSL, and the FA was calculated. The hemisphere ipsilateral to the carotid stenosis was set to the left side by flipping along the mid-sagittal axis. The resulting FA images were fed into TBSS to perform the voxel-wise statistical analysis. All of the FA maps were spatially aligned in a 1×1×1 mm standard MNI to generate a mean FA image. The threshold of the mean FA map was defined as a mean FA value of 0.2 to generate a white matter skeleton mask.

fcMRI analytic preprocessing
The preprocessing procedure included steps to reduce scanner artifacts, correct head motion and transform the data into a standard space. Atlas registration was achieved by joining the
fMRI run with the T1-weighted anatomical images. The correlation maps were generated as previously described: the data were band-pass filtered for frequencies between 0.009 and 0.08 Hz and then spatially smoothed using a 6-mm, full-width, half-maximum Gaussian kernel. The sources of spurious or regionally nonspecific variance were removed, including 6 parameters obtained from the rigid body head motion correction, the signal averaged over the whole brain, the signal averaged over the lateral ventricles and the signal averaged over the deep white matter.
Supplemental Tables

S1. Regions of interest

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Atlas coordinates represent the MNI coordinate system
### S2. Patient characteristics

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* Subjects undergoing CAS; † MRI before and 3 months after CAS with indicated MRI interval in days. The median time between first and 3-month MRI is 95 (interquartile range: 5) days. F: female; M: male; R: right side; L: left side; D: dizziness with DHI>0; A: amnesia; HTN: hypertension; DM: diabetes mellitus; IHD: ischemic heart disease; PAOD: peripheral arterial occlusive disease; C: hypercholesterolemia; S: smoking.
Supplemental References


2. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp. 2002;17:143-155