



Connectometry evaluation in patients undergoing carotid endarterectomy: an exploratory study

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Abstract

This research investigated local brain connectivity changes following Carotid Endarterectomy (CEA) by connectometry. Seventeen subjects (15 males and 2 females, mean age 74.1 years), all eligible for CEA, were prospectively recruited in this exploratory study. On the same day within the week before the CEA, each patient underwent a cognitive evaluation with a Mini Mental State Examination (MMSE) and a Magnetic Resonance Imaging (MRI) exam that included a DTI sequence for the connectometry analysis. A second MMSE and the same MRI protocol were performed on follow-up, 3–6 months after CEA. The MMSE scores were analyzed using T-Student tests. The connectometry analysis was performed using a multiple regression model to consider the effect of CEA, choosing three different T-score threshold (T-threshold) values (1, 2 and 3). Results were considered statistically valid for p value adjusted for False Discovery Rate (p -FDR) < 0.05 . Comparison of pre-CEA and post-CEA MMSE scores showed improvement of MMSE scores after CEA. Connectometry analysis revealed no areas of statistically significant increased connectivity related to CEA for T-threshold value = 1 and 2, but showed statistically significant increase of connectivity after CEA in both cerebellar hemispheres and corpus callosum for T-threshold value = 3 (p -FDR = 0.0106667). The network property analysis showed improved small worldness (2.14%), clustering coefficient (1.64%), local (1.94%) and global efficiency (0.56%), and reduced characteristic path length (−0.52%) after CEA. These results suggest that CEA is associated both with cognitive performance improvement and changes in interhemispheric local connectivity in the corpus callosum and cerebellum.

Keywords Carotid endarterectomy · DTI · Connectometry

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Introduction

Atherosclerotic disease involving the Internal Carotid Artery (ICA) is a well-known risk factor for ischemic stroke (Flaherty et al. 2013; Johnston et al. 2004; Saba et al. 2014, 2018). Similarly, the association between ICA disease and cognitive dysfunction in patients without clinically evident cerebrovascular disease is well established (Dharmakidari et al. 2017). Carotid artery revascularization (either through Carotid Endarterectomy (CEA) or Carotid Artery Stenting (CAS)) was demonstrated to be protective against occurrence of stroke in symptomatic and asymptomatic subjects (Flaherty et al. 2013; Morris et al. 2017).

Several studies using clinical neuropsychological tests demonstrated that CAS (Moftakhar et al. 2005; Grunwald et al. 2006) and CEA (Carta et al. 2015) are associated with increased cognitive performance, and that the improvements is independent from the treatment type (CEA/CAS) (Wapp et al. 2015). Resting State Functional Connectivity Magnetic

Resonance (rs-fcMR) allows to study the brain networks' spontaneous activity while the patient lies inactive in an MRI scanner, analyzing Blood Oxygenation Levels Dependent (BOLD) signal fluctuation generated by local changes of deoxyhemoglobin levels of the active brain networks (Buchbinder 2016). DTI evaluates anisotropic diffusion of water molecules inside of the white matter bundles, in order to estimate axonal organization of the brain (Mori and Zhang 2006), and tractography studies cerebral white matter bundles exploiting DTI principles.

Some of the above mentioned studies analyzed differences in brain connectivity comparing patients with ICA stenosis and normal healthy controls (Lin et al. 2014; Chang et al. 2016; Avirame et al. 2015; Wang et al. 2017a), others investigated brain connectivity before and after CAS (Cheng et al. 2012; Wang et al. 2017b), and one study compared changes following CAS or medical therapy (Lin et al. 2016). Another study by Schaaf et al. (2010) demonstrated increased BOLD signal immediately after CEA probably due to the higher intracranial blood flow.

In the last decade, a new tool for the in-vivo analysis of white matter, called “MRI Connectometry”, has been introduced in research in order to overcome the limitations of conventional tractography approach and DTI (Yeh et al. 2013a, 2016a). Connectometry allows to explore the local connectome, i.e. the degree of connectivity between adjacent voxels of the white matter fascicles according to the density of the diffusing spins (Yeh and Tseng 2011). The technique is based on the reconstruction of the MRI diffusion data into a standard template space, obtaining a map of the local connectome matrix from a group of subjects. The use of a fiber tracking algorithm on this matrix, both with the comparison of the results with a null distribution of coherent associations using permutation statistics, allows to track only the bundles of white matter that show a significant positive or negative correlation with the study variable (Yeh et al. 2016a). The spin distribution function (SDF) is the parameter used for the analysis (Yeh and Tseng 2011). This method has been already used for example for the study of Parkinson disease (Sobhani et al. 2017), mood disorders (Olvet et al. 2016), multiple sclerosis (Romascano et al. 2015) and amyotrophic lateral sclerosis (Abhinav et al. 2014).

The purpose of our study is to identify whether, in asymptomatic patients with severe ICA ($\geq 70\%$) stenosis measured according to North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria (Moneta et al. 1993) treated with CEA, the cognitive function improvement observed in the short-term post-surgical period is associated with changes in brain local connectivity using MRI connectometry, independently from the following conditions: a) mono or bilateral severe ($\geq 70\%$) stenosis and b) presence or absence of cognitive dysfunction.

Materials and methods

Patient enrollment and MRI technique

Our study was designed as a prospective, longitudinal, exploratory study, and it was approved by our ethical committee. Our plan was to recruit at least 12 patients in 1 year (Julious 2005).

Inclusion criteria were as follows:

1. Asymptomatic patients with mono-lateral or bilateral ICA stenosis $\geq 70\%$ documented on CT-angiography according to NASCET (Moneta et al. 1993);
2. All patients had to be eligible for a CEA procedure according to the guidelines of the European Society for Vascular Surgery (ESVS) (Liapis et al. 2009).

Exclusion criteria were as follows:

1. Past medical history of severe systemic, inherited or acquired disease (in particular severe psychiatric/neurological conditions and major stroke), except cognitive impairment;
2. Contraindications to MRI examinations (e.g. non-MRI compatible metallic implants);
3. Functional disability, measured as a modified Rankin' scale score ≥ 2 (van Swieten et al. 1988);
4. Symptomatic patients with history of Transient Ischaemic Attack (TIA), minor stroke or major stroke;
5. Previous CEA/CAS procedure.

All patients recruited gave their written informed consent before their enrollment in the study. Within the week before the surgical intervention all patients took the Italian version of Mini Mental State Examination (MMSE) corrected for age and level of education (Folstein et al. 1975; Magni et al. 1996) for evaluation of their cognitive status. The MMSE was administered by an expert colleague (DC, 9 years of experience). On the same day, a noncontrast MRI scan was performed on a 1.5 Tesla Philips “Achieva dStream” scanner (Philips, Best, Netherland) (peak amplitude 33mT/m, slew rate 160 mT/m/ms) using a 32 channels head coil. The MRI protocol included a DTI sequence with the following parameters: 64 diffusion sampling directions, Echo Time (TE) = 83.147 ms, Repetition Time (TR) = 3370 ms, b-values = 0 and 800 s/mm^2 ; in-plane resolution = 1.75 mm, slice thickness 2.5 mm.

The other sequences included in the MRI protocol were: a) Diffusion Weighted Imaging Single shot (DWI-SSH) sequence, TE = 74 ms, TR = 3546.05 ms, b-values = 0 and 1000 s/mm^2 , slice thickness = 2.5 mm, b) 3D Fluid Attenuated Inversion Recovery (FLAIR), TE = 292.283 ms, TR = 4800 ms, Inversion Time = 1660 ms, Flip Angle 90°,

slice thickness = 1 mm, spacing between slices = 0.57 mm.; c) 3D T1-weighted Turbo Field Echo (TFE), TE = 3.43 ms, TR = 7.5 ms, Flip Angle = 8°, slice thickness = 1 mm, spacing between slices = 1 mm.

Patients with MRI findings of acute lacunar and/or territorial stroke, as well as those with imaging evidence of chronic territorial stroke, were excluded from the study. Patients with small hyper-intense areas on FLAIR sequences suggestive for leukoaraiosis or chronic lacunar strokes were included in the study, because these are common findings in patients with severe ($\geq 70\%$) ICA stenosis (Yamauchi et al. 1999).

Follow-up MMSE and MRI examination, with the same sequences and parameters used in the first MRI evaluation, were performed on the same day, in a period between 3 and 6 months from the surgical intervention (average follow-up time: 4.08 months).

Connectometry analysis

A total of 34 diffusion MRI scans (17 pre-CEA and 17 post-CEA) were included in connectometry database. The analysis of the data was conducted using DSI Studio (release 2017_08 - <http://dsi-studio.labsolver.org>). The diffusion data was reconstructed in the Montreal Neurological Institute (MNI) space using q-space diffeomorphic reconstruction (Yeh and Tseng 2011) to obtain the SDF (Yeh et al. 2010), adopting the Human Connectome Project 1021 (HCP-1021) template as diffusion MRI atlas (Yeh and Tseng 2011). A diffusion sampling length ratio of 1.25 was used, and the output resolution was 1 mm. The restricted diffusion was quantified using restricted diffusion imaging (Yeh et al. 2017). The SDF values were used in the connectometry analysis.

Diffusion MRI connectometry (Yeh et al. 2016a) was used to study the effect of CEA. CEA variable was associated with this local connectome matrix in order to identify those local connectomes that expressed significant association with it.

Three different T-score threshold (T-threshold) values (1, 2 and 3) were assigned in three consecutive analyses in order to select local connectomes, and the local connectomes were tracked along the core pathway of a fiber bundle using a deterministic fiber tracking algorithm and compared with a null distribution of coherent associations using permutation statistics (Yeh et al. 2013b, 2016a; Nichols and Holmes 2002). Track trimming was conducted with 1 iteration. All tracks generated from bootstrap resampling were included. A length threshold of 35 mm was used to select tracks. The seeding density was 40 seeds per mm^3 and a total of 6000 randomized permutations were applied to the group label to obtain the null distribution of the track length. These parameters were chosen in order to improve specificity of the analysis and trying to overcome the limits due to the low number of available cases (even if the research was designed as exploratory).

Statistical analysis

Statistical analysis was performed with the SPSS 24.0 statistical package (SPSS Inc., Chicago, IL). The normality of each continuous variable group was tested using the Kolmogorov-Smirnov normality test. The pre-CEA and post-CEA MMSE scores then were compared using T-Student for paired values test, and a p value <0.05 was regarded to indicate statistically significant association. All p values were calculated using two-tailed significance level.

The connectometry analysis used a multiple regression model to evaluate CEA variable using three different T-threshold values (1, 2 and 3). Permutation test allowed to estimate and correct the false discovery rate (FDR) of Type-I error inflation due to multiple comparisons (Yeh et al. 2016a). p value corrected for FDR (p -FDR) <0.05 was regarded to indicate statistically significant association. The network property analysis was performed analyzing the data from whole brain connectometry database and from tracks positively correlated with CEA, according to the formula:

$$\frac{A-B}{B}$$

where A = values derived by network analysis of whole brain connectometry database and from tracks positively correlated with CEA, and B = values derived by network analysis of whole brain connectometry database. Clustering coefficient, network characteristic path length, global efficiency, local efficiency and small worldness value differences were evaluated as percentage change.

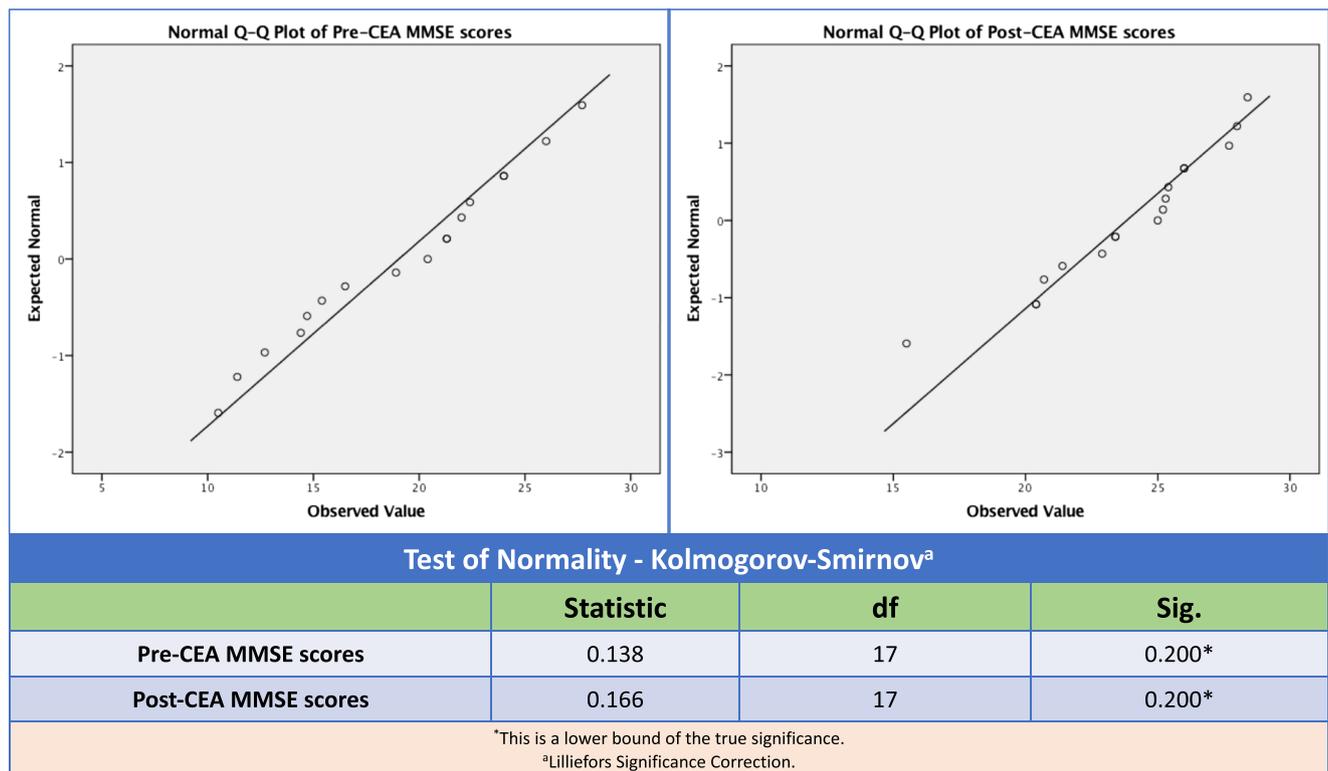
Results

From January 2017 up to December 2017, 17 consecutive patients (15 males and 2 females; age between 65 and 84; mean age: 76.35; mean age for male group: 75.93; mean age for female group: 79.5) were prospectively enrolled in the study (demographic data reported in Table 1). Neither surgical nor post-surgical complications occurred in any patients during their hospital stay. The analysis of follow-up structural MR sequences after CEA did not reveal any new incidental findings, including territorial or lacunar strokes.

The analysis of MMSE examinations revealed that, after the CEA procedure, all the patients improved their cognitive performance (p value = 0.0001), with pre-CEA mean average score of 19.03 and post-CEA mean average score of 23.82 (Figs. 1 and 2).

Table 1 Demographic data

Demographic data							
Patients	Age	Sex	MMSE pre-CEA score	MMSE post-CEA score	Monolateral (M) or bilateral (B) ICA stenosis	Side of ICA obstruction	Follow-up time (months)
Subject 1	76	F	26	26	B	Right	6
Subject 2	83	F	16.5	21.4	B	Right	4
Subject 3	65	M	18.9	22.9	B	Right	3
Subject 4	70	M	20.4	23.4	M	Left	4
Subject 5	71	M	21.3	23.4	B	Right	4
Subject 6	72	M	21.3	25.3	M	Left	4
Subject 7	72	M	27.7	27.7	M	Left	3
Subject 8	73	M	15.4	20.4	M	Left	5
Subject 9	75	M	22	28	M	Left	6
Subject 10	76	M	24	25	M	Right	3
Subject 11	76	M	12.7	20.7	B	Right	4
Subject 12	77	M	24	26	M	Right	5
Subject 13	80	M	22.4	28.4	M	Right	3
Subject 14	81	M	11.4	25.4	M	Left	3
Subject 15	82	M	14.4	20.4	B	Left	4
Subject 16	83	M	10.5	15.5	M	Left	3
Subject 17	84	M	14.7	25.2	M	Right	6
Mean values	76,23	–	19.03	23.82	–	–	4.08

**Fig. 1** Graphs and resume table of the Kolmogorov-Smirnov test that confirmed the normal distribution of both Pre-CEA and Post-CEA MMSE scores. df = degrees of freedom; Sig. = significance

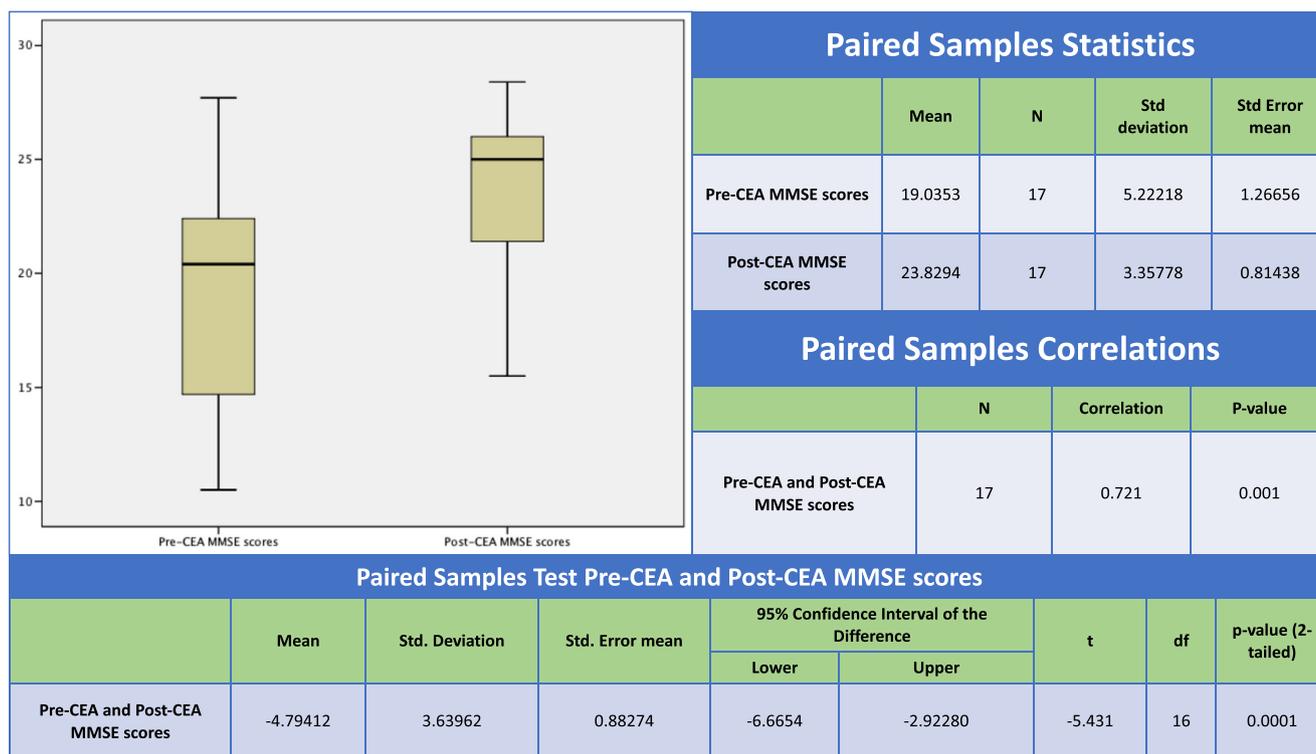


Fig. 2 Graphs and resume table of the T-Student for paired values test, showing improvement of MMSE scores after CEA (p value = 0.0001), with a Pre-CEA mean average score of 19.03 and Post-CEA mean

average score of 23.82. N = number of samples; Std deviation = Standard deviation; Std Error mean = Standard error of the mean; t = t -value; df = degrees of freedom

The connectometry analyses performed using T-score threshold = 1 and 2 did not reveal statistically significant results (p -FDR > 0.05).

The same analysis performed adopting T-score threshold value = 3 showed greater local connectivity after CEA procedure in both the cerebellar hemispheres and corpus callosum (p -FDR = 0.0106667) and no tract with

decreased local connectivity (Figs. 3 and 4); p -FDR trend in relation to fiber length for T-Score threshold = 3 is reported (Fig. 5). The network analysis showed improved clustering coefficient (1.64%) with reduced network characteristic path length (-0.52%), and improved global efficiency (0.57%), local efficiency (1.94%) and small worldness (2.17%).

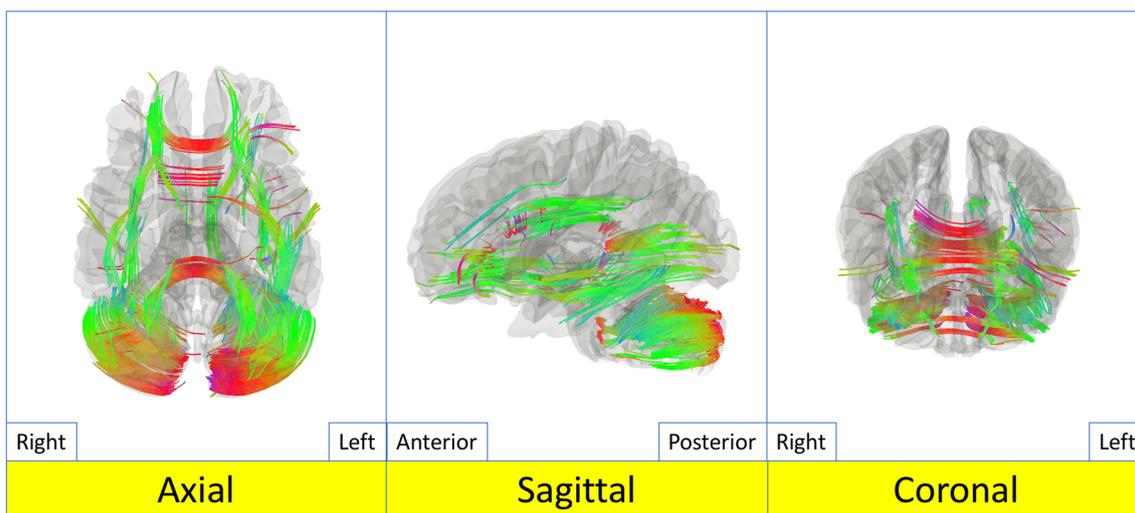


Fig. 3 Brain regions that showed increased local connectivity after CEA procedure. The color of the tract depends on the direction of the fibers (red for right-left, blue for foot-head, green for anterior-posterior)

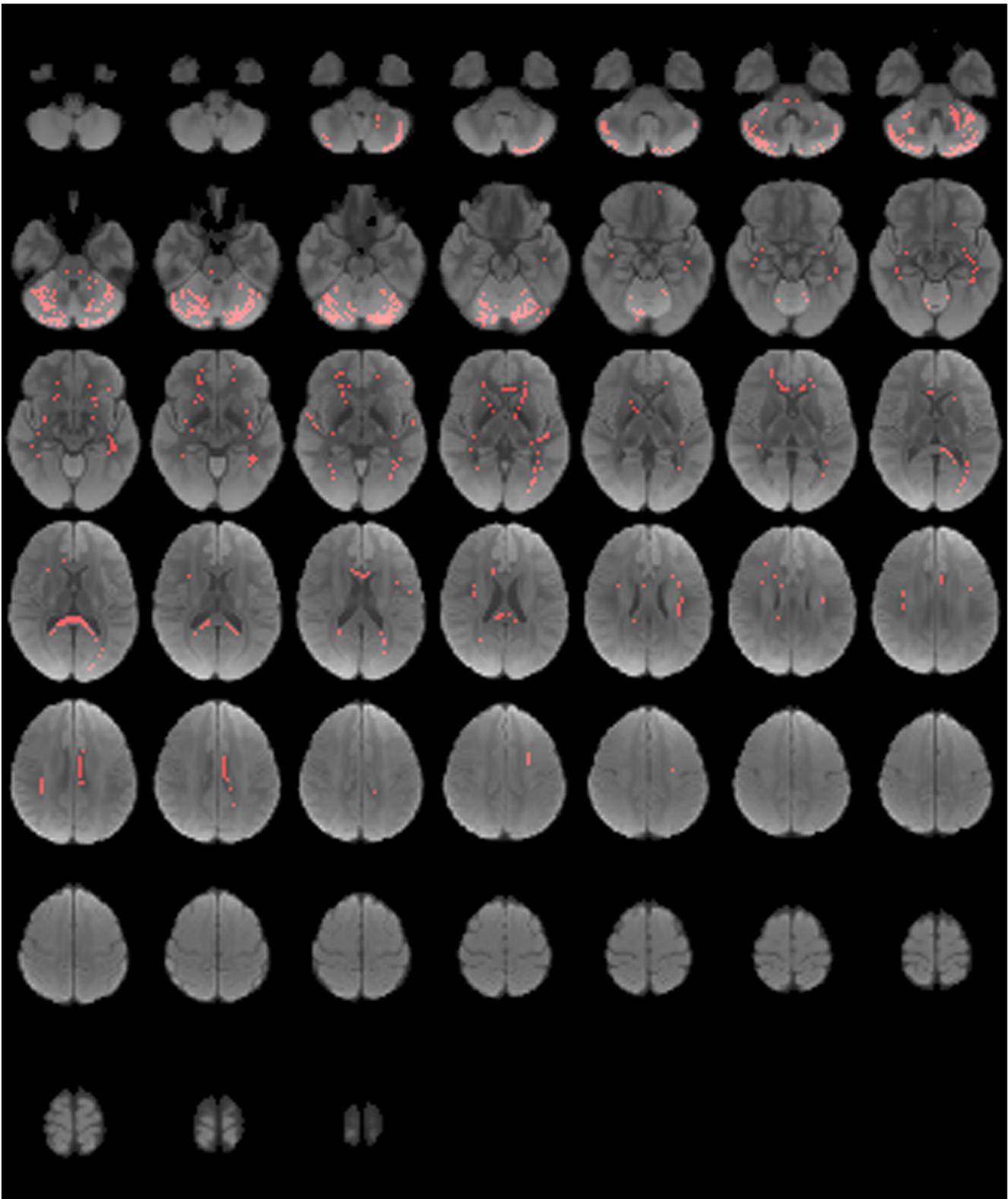


Fig. 4 Mosaic view (radiologic orientation) of the fibers that showed increased local connectivity after CEA procedure (red colour)

The results derived from the network property analysis are reported (Table 2), as well as the mean SDF value of each

subject for corpus callosum and cerebellar hemispheres (Table 3).

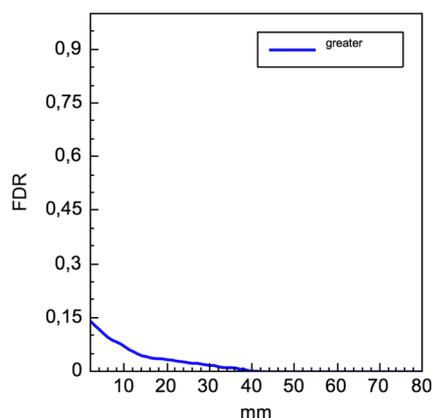


Fig. 5 p-FDR trend (vertical axis) in relation to fiber length (horizontal axis) for T-Score threshold = 3

Discussion

In this analysis, we investigated if short-term brain local connectivity changes after CEA occurred, and if they were accompanied by changes in cognitive performance measured by MMSE. The group connectometry analysis revealed that patients after CEA showed statistically significant increase of local connectivity in corpus callosum and cerebellum when compared to baseline whereas no areas of reduced local connectivity were found. The analyses were performed using three different T-threshold values (1, 2 and 3) similarly to Liu et al. (2018), because of the different impact and significance of the T-threshold values where lower T-threshold values tend to privilege sensitivity whereas higher T-threshold values the specificity. The analysis conducted with T-threshold = 3, where the specificity of the test is maximized, was the only one who gave statistically significant results.

In addition to the connectometry analysis results, the MMSE scores analysis showed statistically significant improvement of MMSE scores after CEA.

The correlation between carotid artery stenosis, cognitive function and brain connectivity impairments was examined in previous studies. Both CAS and CEA are associated with long-term improvements in cognitive performances (Zuniga et al. 2016). Different MRI studies tried to identify brain structural, functional and biochemical differences among patients with asymptomatic ICA stenosis and HCs. Lin et al. (2014) compared a cohort of 30 cognitively intact subject with

asymptomatic, severe ($\geq 70\%$) unilateral ICA stenosis and a second cohort of healthy controls (HCs) using DTI, resting state fMRI, and a comprehensive battery of neuropsychiatric tests: on DTI, the whole brain Fractional Anisotropy (FA), an index linked to the degree of myelination of white matter fibers (Chang et al. 2017), was reduced in patients with asymptomatic unilateral ICA stenosis than HCs; on resting state fMRI, the cohort of asymptomatic patients showed reduced connectivity of the Default Mode Network (DMN), Dorsal Attention Network (DAN), fronto-parietal network and sensorimotor network. A similar approach was adopted by Chang et al. (2016), comparing patients with unilateral ICA stenosis ($\geq 60\%$) and HCs using a battery of neuropsychological tests, resting state fMR and perfusion MR. Network analysis revealed that, in the carotid stenosis patients' group the hemispheres ipsilateral to the ICA stenosis was impaired in "degree" and "global efficiency". Avirame et al. (2015) suggested that cerebral vascular autoregulation, measured as vasomotor reactivity measured by transcranial Doppler, can be one of the mechanisms involved in structural and functional connectivity impairment of cerebral networks in asymptomatic patients with either ICA occlusion or high-grade ICA stenosis. Wang et al. (2017a) compared patients with asymptomatic ICA stenosis and HCs using cognitive tests and an integrated MR approach that consisted of pulsed Arterial Spin Labeling (pASL), proton spectroscopy (MRS) and resting state fMR: this research revealed that ICA stenosis is associated with lower scores at the neurocognitive tests and decreased cerebral blood flow (CBF) in left frontal gyrus, decreased N-Acetyl-Aspartate (NAA) / Creatine (Cr) ratio in the left hippocampus and reduced connectivity in PCC and anterior part of the DMN.

Few imaging longitudinal studies investigated on the effects of ICA revascularization on brain function. In 2010 Schaaf et al. (2010) studied the early effects of CEA on functional MR, revealing that immediately after CEA BOLD signal changes as reflection of ameliorated cerebrovascular reactivity, but in this study only the mean signal-intensity change was assessed. Cheng et al. (2012) compared HCs with patients with $\geq 70\%$ asymptomatic, unilateral internal carotid artery stenosis using a comprehensive neuropsychological battery and a multimodality neuroimaging approach, including DTI and fMR; patients with carotid artery stenosis showed poorer performance in cognitive tests and marked reduction of inter-hemispheric and intra-hemispheric connectivity ipsilateral to carotid stenosis at the level of the fronto-parietal DMN; after successful CAS, small but measurable increments of the mean FA and functional connectivity in the DMN regions were noted. Another study by Lin et al. (2016) analyzed longitudinally two different groups of asymptomatic patients with severe unilateral ICA stenosis ($\geq 70\%$) by using neuropsychological tests, structural MR imaging, DTI and resting state fMR: one group was treated with aggressive medical therapy

Table 2 Effect of CEA on network topology measures

PARAMETERS	VALUE DIFFERENCES %
Clustering coefficient	1.64
Network characteristic path length	-0.52
Global efficiency	0.57
Local efficiency	1.94
Small worldness	2.17

Table 3 Individual mean sdf values of corpus callosum, right and left cerebellum

Subjects	Corpus Callosum		Right Cerebellum		Left Cerebellum	
	PRE-CEA mean SDF value	POST-CEA mean SDF value	PRE-CEA mean SDF value	POST-CEA mean SDF value	PRE-CEA mean SDF value	POST-CEA mean SDF value
Subject 1	0.425932	0.430182	0.401113	0.412328	0.429623	0.442223
Subject 2	0.465825	0.482485	0.468342	0.472416	0.48073	0.480227
Subject 3	0.42844	0.469458	0.381734	0.417663	0.407788	0.449707
Subject 4	0.430207	0.419766	0.382847	0.375684	0.408491	0.395778
Subject 5	0.476657	0.499202	0.48307	0.510129	0.499955	0.534996
Subject 6	0.493297	0.437532	0.43368	0.452917	0.461642	0.462874
Subject 7	0.403378	0.547071	0.400769	0.489578	0.416228	0.535478
Subject 8	0.474237	0.453559	0.489609	0.472007	0.50849	0.485674
Subject 9	0.535694	0.489676	0.492912	0.505739	0.539494	0.529458
Subject 10	0.496544	0.50148	0.4952	0.444496	0.523866	0.490145
Subject 11	0.489673	0.50994	0.410434	0.461874	0.452085	0.516854
Subject 12	0.535235	0.51101	0.542986	0.523238	0.5944	0.555577
Subject 13	0.447937	0.451946	0.461538	0.462228	0.472534	0.474103
Subject 14	0.401145	0.409991	0.40987	0.410676	0.418698	0.431808
Subject 15	0.381989	0.384453	0.386843	0.364996	0.420766	0.395167
Subject 16	0.44937	0.451923	0.43851	0.461721	0.460699	0.487624
Subject 17	0.42003	0.44921	0.430865	0.441706	0.450925	0.458038

alone and the other one with aggressive medical therapy in combination with CAS. Patients were evaluated at baseline and 3 months after treatment: they found that the group treated with aggressive medical therapy and CAS showed a small increase in FA at the splenium of the corpus callosum, an increase functional connectivity of the DAN at the level of insular cortex and of the DMN at the level of MPFC. Recently, Wang et al. (2017b) demonstrated on fcMR that cognitive improvements observed after CAS in asymptomatic patients can be partly correlated with increased connectivity to the posterior cingulate cortex (PCC) in the right SupraFrontal Gyrus (rSFG) on resting state fcMR and increased perfusion in the left frontal gyrus (IFG) on pulsed Arterial Spin Labeling (pASL). To our knowledge, there is no similar study that investigated changes in brain networks activity on fcMR after CEA in patients with severe ICA stenosis.

The study by Cheng et al. (2012), reported above demonstrated that asymptomatic ICA stenosis is associated with reduced interhemispheric connectivity, and it is known that corpus callosum is implied in interhemispheric communication (van der Knaap and van der Ham 2011; Renard et al. 2014). Based on our exploratory study, we can hypothesize that the

augmented local connectivity reflects incremented interactions between cerebral hemispheres.

Regarding the cerebellar findings, the evidence supporting the role of cerebellum in cognitive functions is growing (Buckner 2013; Sullivan 2010). From an evolutionistic point of view, the expansion of the prefrontal region of the brain is associated with volume expansion of cerebellar regions Crus I and II (Balsters et al. 2010); furthermore, cerebellar regions Crus I and II are directly interconnected with prefrontal regions and participate to cognitive processes (Kelly and Strick 2003). Cerebellar changes are not only largely disease-specific, but they are also associated with cortical and subcortical changes in neurodegenerative conditions (Gellersen et al. 2017). In our study, the increased local connectivity of the cerebellar hemispheres following CEA could contribute to the improvement of neurocognitive performances.

The network property analysis evidenced different rearrangements of the network properties (Bullmore and Sporns 2009) following CEA. In particular, our analysis evidenced a slight improvement in clustering coefficient (i.e. the coefficient that quantifies the connections existing between the nearest neighbours of a node) and consequently also in

local efficiency of information transfer and robustness. Further, the observed slightly reduced network characteristic path length (i.e. the minimum number of edges that have to be crossed from one node to another) was reflected by increased global efficiency. Finally, higher clustering and shorter paths contributed to small worldness increase. These network changes could underlie, and partly explain, the cognitive improvements following this surgical procedure.

To the best of our knowledge, this is the first longitudinal study that used connectometry to investigate the mid-term changes in local connectivity after CEA treatment. As opposed to typical fMR analyses that exploit BOLD signal differences to assess the spontaneous brain networks' activity of local brain areas (in particular at the level of grey matter), connectometry allows to analyze the local connectome, i.e. to track the local connectivity patterns along the WM fibers pathways associated with a selected study variable, in our case the CEA procedure. Previously published studies investigated connectometry differences in other neurological diseases (Sobhani et al. 2017; Olvet et al. 2016; Romascano et al. 2015; Abhinav et al. 2014), adopting SDF as biomarker of investigation. SDF is a density-based measurement of diffusion at different orientations. It measures the density of diffusing water, in a different way from other diffusivity measurements such as FA, Apparent Diffusion Coefficient (ADC) or Radial Diffusivity (RD). The reproducibility and uniqueness of SDF is higher than other diffusivity-based measurements: Yeh et al. (2016b) showed that SDF provides a unique structural characterization that can reliably identify single subjects (local connectome fingerprint). Since SDF reveals high individuality, it can be considered a good parameter of inter-subject variance and connectometry is suitable for longitudinal study. We also adopted this analysis technique because connectometry is able to track differences for the whole brain, differently from other track-based or region-based diffusion analyses that compare diffusion data within a given predefined region.

We acknowledge several limitations to our study. The first one is the small number of patients enrolled. However, our main focus was to explore the potential role of connectometry technique in the study of brain changes after CEA in patients with severe ICA stenosis. Future studies with larger sample sizes will be necessary to further expand the model by including additional variables such as vertebral status, mono/bilateral severe stenosis and/or presence/absence of cognitive dysfunction.

Another limitation is the use of MMSE as the only test for the evaluation of the patients' cognitive function: previous studies demonstrated that patients who underwent CEA showed improvements in cognition, mood and quality of life tests (Grunwald et al. 2006; Liu et al. 2018). Even if MMSE is not the optimal test for cognitive analysis in patients

with ICA stenosis, it was used as surrogate test to give general indications on the trend for neurocognitive performance before and after the surgical procedure in this exploratory study.

The MRI technique could represent another limitation. The majority of connectometry studies were performed using 3.0 Tesla MR scanners and b-values higher than 800 mm/s²; our intent was to investigate the potential role of the connectometry technique in the setting of routine clinical practice, using relative fast sequences easy to be incorporated in a standard MR scan protocol.

Lastly, our limited understanding of the mechanisms underlying neurocognition in normal healthy subjects, and the relatively recent introduction of the connectometry technique for the study of neurological diseases limit the interpretation of our results, and future similar investigations on healthy and diseased subjects are required.

Conclusions

This exploratory research investigated the short-term effects of CEA on neurocognitive performance using connectometry in asymptomatic patients eligible for CEA. The results obtained suggest that the cognitive improvement observed after CEA could be related to increased local interhemispheric connectivity and cerebellar local connectivity. Our results could represent a starting point to re-think the role of carotid revascularization not only for stroke prevention, but also for cognitive improvement in selected patients with cognitive impairment.

Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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References

- Abhinav, K., Yeh, F. C., El-Dokla, A., Ferrando, L. M., Chang, Y. F., Lacomis, D., Friedlander, R. M., & Fernandez-Miranda, J. C. (2014). Use of diffusion spectrum imaging in preliminary

- longitudinal evaluation of amyotrophic lateral sclerosis: development of an imaging biomarker. *Frontiers in Human Neuroscience*, 8, 270.
- Avirame, K., Lesemann, A., List, J., Witte, A. V., Schreiber, S. J., & Flöel, A. (2015). Cerebral autoregulation and brain networks in occlusive processes of the internal carotid artery. *Journal of Cerebral Blood Flow & Metabolism*, 35(2), 240–247.
- Balsters, J. H., Cussans, E., Diedrichsen, J., Phillips, K. A., Preuss, T. M., Rilling, J. K., & Ramnani, N. (2010). Evolution of the cerebellar cortex: the selective expansion of prefrontal-projecting cerebellar lobules. *NeuroImage*, 49(3), 2045–2052.
- Buchbinder, B. R. (2016). Functional magnetic resonance imaging. *Handbook of Clinical Neurology*, 135, 61–92.
- Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron*, 80(3), 807–815.
- Bullmore, E., & Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nature Reviews Neuroscience*, 10(3), 186–198 Erratum in: *Nat Rev Neurosci*. 10(4):312.
- Carta, M. G., Lecca, M. E., Saba, L., Sanfilippo, R., Pintus, E., Cadoni, M., Sancassiani, F., Moro, M. F., Craobedda, D., Lo Giudice, C., Finco, G., Musu, M., & Montisci, R. (2015). Patients with carotid atherosclerosis who underwent or did not undergo carotid endarterectomy: outcome on mood, cognition and quality of life. *BMC Psychiatry*, 15, 277.
- Chang, T. Y., Huang, K. L., Ho, M. Y., Ho, P. S., Chang, C. H., Liu, C. H., Chang, Y. J., Wong, H. F., Hsieh, I. C., Lee, T. H., & Liu, H. L. (2016). Graph theoretical analysis of functional networks and its relationship to cognitive decline in patients with carotid stenosis. *Journal of Cerebral Blood Flow and Metabolism*, 36(4), 808–818.
- Chang, E. H., Argyelan, M., Aggarwal, M., Chandon, T. S., Karlsgodt, K. H., Mori, S., & Malhotra, A. K. (2017). The role of myelination in measures of white matter integrity: combination of diffusion tensor imaging and two-photon microscopy of CLARITY intact brains. *NeuroImage*, 147, 253–261.
- Cheng, H. L., Lin, C. J., Soong, B. W., Wang, P. N., Chang, F. C., Wu, Y. T., Chou, K. H., Lin, C. P., Tu, P. C., & Lee, I. H. (2012). Impairments in cognitive function and brain connectivity in severe asymptomatic carotid stenosis. *Stroke*, 43(10), 2567–2573.
- Dharmakidari, S., Bhattacharya, P., & Chaturvedi, S. (2017). Carotid artery stenosis: medical therapy, surgery. and Stenting. *Current Neurology and Neuroscience Reports*, 17(10), 1–7.
- Flaherty, M. L., Kissela, B., Khoury, J. C., Alwell, K., Moomaw, C. J., Woo, D., Khatri, P., Ferioli, S., Adeoye, O., Broderick, J. P., & Kleindorfer, D. (2013). Carotid artery stenosis as a cause of stroke. *Neuroepidemiology*, 40(1), 36–41.
- Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, 12(3), 189–198.
- Gellersen, H. M., Guo, C. C., O'Callaghan, C., Tan, R. H., Sami, S., & Homberger, M. (2017). Cerebellar atrophy in neurodegeneration—a meta-analysis. *Journal of Neurology, Neurosurgery, and Psychiatry*, 88(9), 780–788.
- Grunwald, I. Q., Suppran, T., Politi, M., Struffert, T., Falkai, P., Krick, C., Backens, M., & Reith, W. (2006). Cognitive changes after carotid artery stenting. *Neuroradiology*, 48(5), 319–323.
- Johnston, S. C., O'Meara, E. S., Manolio, T. A., Lefkowitz, D., O'Leary, D. H., Goldstein, S., Carlson, M. C., Fried, L. P., & Longstreth, W. T., Jr. (2004). Cognitive impairment and decline are associated with carotid artery disease in patients without clinically evident cerebrovascular disease. *Annals of Internal Medicine*, 140(4), 237–247.
- Julious, S. A. (2005). Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics*, 4, 287–291.
- Kelly, R. M., & Strick, P. L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *The Journal of Neuroscience*, 23(23), 8432–8444.
- Liapis, C. D., Bell, P. R., Mikhailidis, D., Sivenius, J., Nicolaidis, A., Fernandes e Fernandes, J., Biasi, G., Norgren, L., & ESVS Guidelines Collaborators. (2009). Invasive treatment for carotid stenosis: Indications, techniques. *European Journal of Vascular and Endovascular Surgery*, 37(4 Suppl), 1–19.
- Lin, C. J., Tu, P. C., Chern, C. M., Hsiao, F. J., Chang, F. C., Cheng, H. L., Tang, C. W., Lee, Y. C., Chen, W. T., & Lee, I. H. (2014). Connectivity features for identifying cognitive impairment in Presymptomatic carotid stenosis. *PLoS One*, 9(1), e85441.
- Lin, C. J., Chang, F. C., Chou, K. H., Tu, P. C., Lee, Y. H., Lin, C. P., Wang, P. N., & Lee, I. H. (2016). Intervention versus aggressive medical therapy for cognition in severe asymptomatic carotid stenosis. *AJNR. American Journal of Neuroradiology*. 2016 Apr 28. [Epub ahead of print].
- Liu, X., Gao, X., Zhang, L., Yuan, Z., Zhang, C., Lu, W., Cui, D., Zheng, F., Qiu, J., & Xie, J. (2018). Age-related changes in fiber tracts in healthy adult brains: a generalized q-sampling and connectometry study. *Journal of Magnetic Resonance Imaging*, 48(2), 369–381.
- Magni, E., Binetti, G., Bianchetti, A., Rozzini, R., & Trabucchi, M. (1996). Mini-mental state examination: a normative study in Italian elderly population. *European Journal of Neurology*, 3(3), 198–202.
- Moftakhar, R., Turk, A. S., Niemann, D. B., Hussain, S., Rajpal, S., Cook, T., Geraghty, M., Aagaard-Kienitz, B., Turski, P. A., & Newman, G. C. (2005). Effects of carotid or vertebrobasilar stent placement on cerebral perfusion and cognition. *AJNR. American Journal of Neuroradiology*, 26(7), 1772–1780.
- Moneta, G. L., Edwards, J. M., Chitwood, R. W., Taylor, L. M., Jr., Lee, R. W., Cummings, C. A., & Porter, J. M. (1993). Correlation of north American symptomatic carotid endarterectomy trial (NASCET) angiographic definition of 70% to 99% internal carotid artery stenosis with duplex scanning. *Journal of Vascular Surgery*, 17(1), 152–157.
- Mori, S., & Zhang, J. (2006). Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*, 51(5), 527–539.
- Morris, D. R., Ayabe, K., Inoue, T., Sakai, N., Bulbulia, R., Halliday, A., & Goto, S. (2017). Evidence-based carotid interventions for stroke prevention: state-of-the-art review. *Journal of Atherosclerosis and Thrombosis*, 24(4), 373–387.
- Nichols, T. E., & Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Human Brain Mapping*, 15(1), 1–25.
- Olvet, D. M., Delaparte, L., Yeh, F. C., DeLorenzo, C., McGrath, P. J., Weissman, M. M., Adams, P., Fava, M., Deckersbach, T., McInnis, M. G., Carmody, T. J., Cooper, C. M., Kurian, B. T., Lu, H., Toups, M. S., Trivedi, M. H., & Parsey, R. V. (2016). A comprehensive examination of white matter tracts and connectometry in major depressive disorder. *Depression and Anxiety*, 33(1), 56–65.
- Renard, D., Castelnovo, G., Campello, C., Bouly, S., Le Floch, A., Thouvenot, E., Waconge, A., & Taieb, G. (2014). An MRI review of acquired corpus callosum lesions. *Journal of Neurology, Neurosurgery, and Psychiatry*, 85(9), 1041–1048.
- Romascano, D., Meskaldji, D. E., Bonnier, G., Simioni, S., Rotzinger, D., Lin, Y. C., Menegaz, G., Roche, A., Schlupe, M., Pasquier, R. D., Richiardi, J., Van De Ville, D., Daducci, A., Sumpf, T., Frahm, J., Thiran, J. P., Krueger, G., & Granziera, C. (2015). Multicontrast connectometry: a new tool to assess cerebellum alterations in early relapsing-remitting multiple sclerosis. *Human Brain Mapping*, 36(4), 1609–1619.
- Saba, L., Anzidei, M., Marincola, B. C., Piga, M., Raz, E., Bassareo, P. P., Napoli, A., Mannelli, L., Catalano, C., & Wintermark, M. (2014).

- Imaging of the carotid artery vulnerable plaque. *Cardiovascular and Interventional Radiology*, 37(3), 572–585.
- Saba, L., Yuan, C., Hatsukami, T. S., Balu, N., Qiao, Y., DeMarco, J. K., Saam, T., Moody, A. R., Li, D., Matouk, C. C., Johnson, M. H., Jäger, H. R., Mossa-Basha, M., Kooi, M. E., Fan, Z., Saloner, D., Wintermark, M., Mikulis, D. J., & Wasserman, B. A. (2018). Vessel wall imaging study group of the American society of neuroradiology. Carotid artery wall imaging: perspective and guidelines from the ASNR vessel wall imaging study group and expert consensus recommendations of the American society of neuroradiology. *AJNR. American Journal of Neuroradiology*, 39(2), E9–E31.
- Schaaf, M., Mommertz, G., Ludolph, A., Geibprasert, S., Mühlenbruch, G., Das, M., & Krings, T. (2010). Functional MR imaging in patients with carotid artery stenosis before and after revascularization. *AJNR. American Journal of Neuroradiology*, 31(10), 1791–1798.
- Sobhani S, Rahmani F, Aarabi MH, Sadr AV (2017) Exploring white matter microstructure and olfaction dysfunction in early parkinson disease: diffusion MRI reveals new insight. *Brain Imaging and Behavior*. 2017 Nov 13. <https://doi.org/10.1007/s11682-017-9781-0>. [Epub ahead of print].
- Sullivan, E. V. (2010). Cognitive functions of the cerebellum. *Neuropsychology Review*, 20(3), 227–228.
- van der Knaap, L. J., & van der Ham, I. J. (2011). How does the corpus callosum mediate interhemispheric transfer? A review. *Behavioural Brain Research*, 223(1), 211–221.
- van Swieten, J. C., Koudstaal, P. J., Visser, M. C., Schouten, H. J., & Gijn, J. (1988). Interobserver agreement for the assessment of handicap in stroke patients. *Stroke*, 19(5), 604–607.
- Wang, T., Xiao, F., Wu, G., Fang, J., Sun, Z., Feng, H., Zhang, J., & Xu, H. (2017a). Impairments in brain perfusion, metabolites, functional connectivity, and cognition in severe asymptomatic carotid stenosis patients: an integrated MRI study. *Neural Plasticity*, 2017, 8738714.
- Wang, T., Sun, D., Liu, Y., Mei, B., Li, H., Zhang, S., & Zhang, J. (2017b). The impact of carotid artery stenting on cerebral perfusion, functional connectivity, and cognition in severe asymptomatic carotid stenosis patients. *Frontiers in Neurology*, 8, 403.
- Wapp, M., Everts, R., Burren, Y., Kellner-Weldon, F., El-Koussy, M., Wiest, R., Federspiel, A., Michel, P., & Schroth, G. (2015). Cognitive improvement in patients with carotid stenosis is independent of treatment type. *Swiss Medical Weekly*, 145, w14226.
- Yamauchi, H., Fukuyama, H., Nagahama, Y., Shiozaki, T., Nishizawa, S., Konishi, J., Shio, H., & Kimura, J. (1999). Brain arteriolosclerosis and hemodynamic disturbance may induce leukoaraiosis. *Neurology*, 53(8), 1833–1838.
- Yeh, F. C., & Tseng, W. Y. (2011). NTU-90: a high angular resolution brain atlas constructed by q-space diffeomorphic reconstruction. *NeuroImage*, 58(1), 91–99.
- Yeh, F. C., Wedeen, V. J., & Tseng, W. Y. (2010). Generalized q-sampling imaging. *IEEE Transactions on Medical Imaging*, 29(9), 1626–1635.
- Yeh, F. C., Tang, P. F., & Tseng, W. Y. (2013a). Diffusion MRI connectometry automatically reveals affected fiber pathways in individuals with chronic stroke. *NeuroImage Clinical*, 2, 912–921.
- Yeh, F.-C., Verstynen, T. D., Wang, Y., Fernández-Miranda, J. C., & Tseng, W.-Y. I. (2013b). Deterministic diffusion fiber tracking improved by quantitative anisotropy. *Zhan W, PLoS One*, 8(11), e80713.
- Yeh, F. C., Badre, D., & Verstynen, T. (2016a). Connectometry: a statistical approach harnessing the analytical potential of the local connectome. *NeuroImage*, 125, 162–171.
- Yeh, F. C., Vettel, J. M., Singh, A., Poczos, B., Grafton, S. T., Erickson, K. I., Tseng, W. I., & Verstynen, T. D. (2016b). Quantifying differences and Similarities in whole-brain white matter architecture using local connectome fingerprints. *PLoS Computational Biology*, 12(11), e1005203.
- Yeh, F. C., Liu, L., Hitchens, T. K., & Wu, Y. L. (2017). Mapping immune cell infiltration using restricted diffusion MRI. *Magnetic Resonance in Medicine*, 77(2), 603–612.
- Zuniga, M. C., Tran, T. B., Baughman, B. D., Raghuraman, G., Hitchner, E., Rosen, A., & Zhou, W. (2016). A prospective evaluation of systemic biomarkers and cognitive function associated with carotid revascularization. *Annals of Surgery*, 264(4), 659–665.