Connectometry evaluation in patients undergoing carotid endarterectomy: an exploratory study.

Keywords

Endarterectomy; DTI; Connectometry.

Abstract

Introduction: Investigating on brain local connectivity changes following Carotid Endarterectomy (CEA) by connectometry.

Methods: In this exploratory study, seventeen subjects (15 males and 2 females, mean age 74.1 years) who underwent CEA, were prospectively recruited. Within one week before the CEA, each patient performed, in the same day, a cognitive evaluation with a Mini Mental State Examination (MMSE) and a Magnetic Resonance (MR) exam that included a DTI sequence for the connectometry analysis. The same cognitive test and MR protocol were performed on follow-up in a period between 3-6 months after CEA. The MMSE scores were analyzed using T-Student test. The connectometry analysis was performed using a multiple regression model in order to consider the effect of CEA, choosing three different T-score threshold values (1, 2 and 3), and results were considered statistically valid when p-value adjusted for False Discovery Rate (p-FDR) < 0.05.

Results: Comparison of pre-CEA and post-CEA MMSE scores showed improvement of MMSE scores after CEA (p-value = 0.0001). Connectometry analysis revealed no areas of statistically significant increased connectivity related to CEA for T-threshold value = 1 and 2, whereas for T-threshold value = 3 the analysis revealed statistically significant increased connectivity after CEA (p-FDR = 0.0106667) in both cerebellar hemispheres and corpus callosum.

Conclusion: The results suggest that CEA procedure is associated with both improvements of cognitive performances and changes in both interhemispheric local connectivity through corpus callosum and in cerebellum.

Introduction

Atherosclerotic disease involving Internal Carotid Artery (ICA) is a well-known risk factor for ischemic stroke [1-3], but also the association between ICA disease and cognitive dysfunctions in patients without clinically evident cerebrovascular disease is well known [4]. In the past years, carotid artery revascularization (following Carotid Endarterectomy (CEA) or Carotid Artery Stenting (CAS)) demonstrated to be protective against occurrence of stroke in symptomatic and asymptomatic subjects [5,6].

Further, several studies investigated on variations in cognitive performance using clinical neuropsychological tests, demonstrating that CAS [7,8] and CEA [9] are associated with increasing in cognitive performances, and these improvements are independent from the treatment type (CEA vs CAS) [10]. Resting State Functional Connectivity Magnetic Resonance (rs-fcMR) allows to study the spontaneous brain networks' activity while patient lies inactive in MR scanner, analyzing Blood Oxygenation Levels Dependent (BOLD) signal fluctuation generated by local changes of deoxyhemoglobin levels of the active brain regions following their activation [11]. DTI allows to evaluate anisotropic diffusion of water molecules inside of the white matter bundles, in order to estimate axonal organization of the brain [12], and tractography allows to study cerebral white matter bundles exploiting DTI principles.

Some of the studies above mentioned analyzed differences in brain connectivity comparing patients with ICA stenosis and normal healthy controls [13-16], others investigated on brain connectivity before and after CAS [17,18], and another one compared changes following CAS or aggressive medical therapy [19]. Another study by *Schaaf et al.* [20] evidenced 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 [21,22]. 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 [23]. 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 shows a significant positive or negative correlation with the study variable [21]. The spin distribution function (SDF) is the parameter used for the analysis [23]. This method has been already used for example for the study of Parkinson disease [24-30], mood disorders [31,32], multiple sclerosis [33] and amyotrophic lateral sclerosis [34].

The purpose of our study is to identify whether in asymptomatic patients with severe ICA (≥ 70%) stenosis measured according to European Carotid Surgery Trial (ECST) criteria [35] treated with CEA, the cognitive improvements registered in the short-term post-surgical period were also associated with changes in brain local connectivity using MR connectometry, independently from these conditions: a) mono or bilateral severe (≥ 70%) stenosis and b) presence or absence of cognitive dysfunction.

Materials and Methods

Patient enrollment and MR examination technique

The study was designed as a prospective study, and it obtained the approval from the ethical committee. According to the exploratory intent, it was planned to recruit in one year at least 12 patients [36]; from January 2017 up to December 2017 at the University Hospital of Cagliari 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). All the patients had to be eligible for monolateral or bilateral CEA procedure according to the guidelines of the European Society for Vascular Surgery [35].

The exclusion criteria were the following:

1. Anamnestic history of severe systemic inherited or acquired disease (in particular severe psychiatric/neurological conditions and major stroke), except cognitive dysfunction.
2. Presence of contraindications to MR examinations (e.g. not compatible metal implants).
3. Presence of functional disability, measured with the modified Rankin' scale (values ≥ 2) [37].
4. Symptomatic patients with history of Transient Ischaemic Attack (TIA), Minor stroke or stroke.

All patients recruited signed the informed consent before their enrollment in the study. All patients within the week before the surgical intervention performed the italian version of Mini Mental State Examination (MMSE) corrected for age and schooling [38,39] for evaluation of cognitive status administered by an expert colleague (DC, 9 years of experience). In the same day a non-contrast resting-state head MR scan was performed with 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 dedicated MR scan protocol included a DTI sequence with the following parameters: 64 diffusion sampling directions, Echo Time (TE) = 83.147ms, 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 MR protocol scan 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; the other exclusion criteria were the presence of incidental findings suggestive for intra-axial or extra-axial neoplastic lesions, inflammatory or infective diseases. On the contrary, patients with small hyperintense areas on FLAIR sequences suggestive for leukoaraiosis/chronic lacunar strokes were included in the study, due to the fact that usually they are common findings in patients with ICA severe (≥ 70%) stenosis and often clinically asymptomatic [40] and the study was designed as longitudinal. No surgical neither post-surgical complications occurred to any patients during hospitalization.

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

Connectometry analysis

Regarding the analysis of imaging investigations, 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 were reconstructed in the Montreal Neurological Institute (MNI) space using q-space diffeomorphic reconstruction [23] to obtain the SDF [41], adopting the Human Connectome Project 1021 (HCP-

1021) template as diffusion MRI atlas [23]. 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 [42]. The SDF values were used in the connectometry analysis.

Diffusion MRI connectometry [21] 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 associations with it.

Three different T-score 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 [21,43,44]. 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-score 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 [21]. p-value corrected for FDR (p-FDR) < 0.05 was regarded to indicate statistically significant association.

Results

None of the subject suffered surgical or medical complications after the procedure. The analysis of follow-up structural MR sequences after CEA did not reveal any new incidental findings, included territorial or lacunar strokes.

The analysis of MMSE examinations revealed that after 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 (Figure 1 and 2).

The connectometry analyses performed using T-score threshold = 1 and 2 did not reveal statistically significant results. 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 tracts with decreased local connectivity (Figure 3 and 4). The mean SDF value of each subject for corpus callosum and cerebellar hemispheres were also reported (Table 2).

Discussion

The correlation between carotid artery stenosis, cognitive function and brain connectivity impairments was described in previous researches. For example, it is known that both CAS and CEA are associated with long-term improvements in cognitive performances [45].

Different MR studies tried to identify brain structural, functional and biochemical differences among patients with asymptomatic ICA stenosis and HCs; in particular *Lin CJ et al.* [13] compared a cohort of 30 cognitively intact subject with asymptomatic, severe (≥ 70%) unilateral ICA stenosis and a second cohort of healthy controls (HCs) using DTI, resting state fcMR, and a comprehensive battery of neuropsychiatric tests: on DTI that the whole brain Fractional Anisotropy (FA), an index supposed to be directly correlated to the degree of myelination of white matter fibers [46], was reduced in patients with asymptomatic unilateral ICA stenosis than HCs; on resting state fcMR 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 TY et al.* [14], comparing patients with unilateral ICA stenosis (≥ 60%) and HCs using a battery of neuropsychological tests, resting state fcMR and perfusion MR. Network analysis revealed that in the patients' group the hemispheres ipsilateral to the ICA stenosis was impaired in "degree" and "global efficiency". *Avirame K et al.* [15] suggested that cerebral vascular autoregulation, in terms of 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. A research by *Wang T et al.* [16] 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 fcMR: this research revealed that the condition of ICA stenosis was 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 longitudinal studies investigated on the effects of ICA revascularization. In 2010 *Schaaf M. et al.* [20] studied the early effects of CEA on functional MR, evidencing that immediately after CEA BOLD signal changes as reflection of ameliorated cerebrovascular reactivity, but in this paper the only mean signal-intensity change was assessed. *Cheng HL et al.* [17] compared HCs with patients with ≥ 70% asymptomatic stenosis of unilateral internal carotid artery using a comprehensive neuropsychological battery and a multimodality neuroimaging approach, that included DTI and fcMR; patients with carotid artery stenosis showed poorer performances 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. Recently *Wang T et al.* [18] 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 functional connectivity Magnetic Resonance (fcMR) and increased perfusion in the left frontal gyrus (lFG) pulsed Arterial Spin Labeling (pASL) that underwent CAS procedure. To our knowledge, there are not similar studies that investigated brain networks activity changes on fcMR after ECA in patients with severe ICA stenosis. Another study by *Lin CJ et al.* [19] analyzed longitudinally two different groups of asymptomatic patients with severe unilateral ICA stenosis (≥ 70%) by using neuropsychological tests, structural MR imaging, DTI and resting state fcMR: one group was treated with aggressive medical therapy alone and the other one with aggressive medical therapy in combination with CAS. Patients were evaluated at the baseline and three months after treatment: they found that 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.

In this analysis, we investigated if there were short-term brain local connectivity changes after CEA, and if they were accompanied or not by changes in cognitive performances measured by MMSE. The group connectometry analysis revealed that patients who underwent CEA showed statistically significant (p-FDR = 0.0106667) short term local connectivity changes in corpus callosum and cerebellum. a statistically significant greater connectivity when compared to the baseline; no areas of reduced local connectivity were found. The Pre-CEA and Post-CEA MMSE scores analysis showed a statistically significant improvement of MMSE scores after CEA (p-value = 0.0001), with Pre-CEA mean average score of 19.03 and Post-CEA mean average score of 23.82.

To the best of our knowledge, this is the first longitudinal study that used the connectometry technique in order investigate on the mid-term local connectivity changes after ICA treatment. Differently from fcMR that analyzes the spontaneous brain networks' activity of local brain areas (in particular at the level of grey matter) exploiting the BOLD signal differences, the connectometry technique allows to analyze the local connectome, i.e. to track the local connectivity patterns along the WM fibers pathways associated with a determine study variable, in this case CEA procedure.

As indicated above, previously published studies has investigated connectometry differences in other neurological diseases [24-34], adopting SDF as biomarker of investigation. SDF is a density-based measurement of diffusion at different orientations and it measures the density of diffusing water, differently from other diffusivity measurements such as Fractional Anisotropy (FA), Apparent Diffusion Coefficient (ADC) and Radial Diffusivity. The reproducibility and uniqueness of SDF is higher than other diffusivity-based measurements: a paper by *Yeh F-C et al.* [47] in fact 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 can be considered suitable for longitudinal study. We adopted this analysis technique also because differently from other track-based or region-based diffusion analysis that compare diffusion data within a given region, connectometry technique is able to track the differences in the whole brain.

The results of our connectometry analysis reveal that following CEA procedure, corpus callosum and both the cerebellar hemispheres show incremented local connectivity.

The study by *Cheng TL et al.* [17], reported above, evidenced that asymptomatic ICA stenosis is associated with reduced interhemispheric connectivity, and it is known that corpus callosum is implied in interhemispheric communication [48,49]. We can speculate that the augmented local connectivity reflects incremented interactions between cerebral hemispheres.

Regarding the cerebellar findings, it is noteworthy the fact that the proofs on the role of cerebellum in cognitive functions are growing up [50,51]. 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 [52]; further it is known that cerebellar regions Crus I and II are directly interconnected with prefrontal regions and participate to cognitive processes [53] and it is also known that cerebellar changes are not only largely disease-specific, but they are also associated with cortical and subcortical changes in neurodegenerative conditions [54]. Also in this case, the incremented local connectivity of the cerebellar hemispheres following CEA could be implied in the improvement of neurocognitive performances.

The low number of longitudinal studies on the effects of ICA revascularization, the absence of a complete knowledge 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 the results, and further similar investigations on healthy and diseased subjects need to be done.

Anyway, to our knowledge, this research both with other similar researches that analyze the effects of carotid revascularization on cognitive performances and cerebral networks rearrangements, could represent starting points to re-think the idea of carotid revascularization not only for prevention of stroke, but also for therapeutic intents in selected patients with cognitive impairment.

In this study there are some limitations. The first one is the small number of patients: our main focus was to explore the potential role of connectometry technique in the study of brain variation after CEA in patient with severe carotid artery stenosis; also with the small cohort considered, our analysis gave statistically significant values (p-FDR = 0.0106667) that allows us to confirm the validity and the strength of this model and results. However further studies with bigger cohort are necessary to further expand the model by including more variables such as the mono/bilateral severe stenosis and or presence/absence of cognitive dysfunction.

Another limit is the use of MMSE as the only test used for the evaluation of the cognitive functions of the patients: cognitive improvements seen after CEA were already demonstrated in previous researches that patients who underwent CEA showed improvements in cognition, mood and quality of life tests [8,45]. Even if MMSE is not the most appropriate test for cognitive analysis in patients with ICA stenosis, it was used as surrogate test in order to give general indications on the trend of neurocognitive performances before and after the surgical procedure due the exploratory intent of the study.

MR technique adopted could represent another limit; the majority of connectometry study have been performed using 3.0 Tesla MR scanners and b-values higher than 800 mm/s^2; our intent was to investigate on the potential role of the connectometry technique also in everyday clinical practice, using relative fast sequences easy to be included in a standard MR scan protocol, and this is also the reason why we did not perform a fcMR study on the same patients.

Conclusions

This exploratory research investigated on the short-term effects of CEA on neurocognitive performances and brain using connectometry in asymptomatic patients eligible for CEA. The results obtained suggest that the cognitive improvement observed can be related to increased local interhemispheric connectivity cerebellar local connectivity, independently from the cognitive status and the condition of mono or bilateral ICA stenosis.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Dr. Michele Porcu wants to thank the members of the department of Diagnostic Imaging of the University Hospital "Duilio Casula" (Monserrato, Cagliari, Italy) for their invaluable support.

References:

1. Flaherty ML, Kissela B, Khoury JC, et al. Carotid Artery Stenosis as a Cause of Stroke. Neuroepidemiology. 2013;40(1):36-41. doi:10.1159/000341410. Johnston SC, O'Meara ES, Manolio TA, Lefkowitz D, O'Leary DH, Goldstein S, Carlson MC, Fried LP, Longstreth WT Jr. Cognitive impairment and decline are associated with carotid artery disease in patients without clinically evident cerebrovascular disease. Ann Intern Med. 2004 Feb 17;140(4):237-47. PubMed PMID: 14970146.
2. Saba L, Yuan C, Hatsukami TS, Balu N, Qiao Y, DeMarco JK, Saam T, Moody AR, Li D, Matouk CC, Johnson MH, Jäger HR, Mossa-Basha M, Kooi ME, Fan Z, Saloner D, Wintermark M, Mikulis DJ, Wasserman BA; 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 Am J Neuroradiol. 2018 Jan 11. doi: 10.3174/ajnr.A5488. [Epub ahead of print] PubMed PMID:29326139.
3. Saba L, Anzidei M, Marincola BC, Piga M, Raz E, Bassareo PP, Napoli A, Mannelli L, Catalano C, Wintermark M. Imaging of the carotid artery vulnerable plaque. Cardiovasc Intervent Radiol. 2014 Jun;37(3):572-85. doi: 10.1007/s00270-013-0711-2. Epub 2013 Aug 3. Review. PubMed PMID: 23912494.
4. Dharmakidari, S., Bhattacharya, P., & Chaturvedi, S. (2017). Carotid Artery Stenosis: Medical Therapy, Surgery, and Stenting. *Current Neurology and Neuroscience Reports*, *17*(10), 1–7. https://doi.org/10.1007/s11910-017-0786-2
5. Morris DR, Ayabe K, Inoue T, et al. Evidence-Based Carotid Interventions for Stroke Prevention: State-of-the-art Review. Journal of Atherosclerosis and Thrombosis. 2017;24(4):373-387. doi:10.5551/jat.38745.
6. Flaherty ML, Kissela B, Khoury JC, et al. Carotid Artery Stenosis as a Cause of Stroke. Neuroepidemiology. 2013;40(1):36-41. doi:10.1159/000341410.
7. Moftakhar R, Turk AS, Niemann DB, Hussain S, Rajpal S, Cook T, Geraghty M, Aagaard-Kienitz B, Turski PA, Newman GC. Effects of carotid or vertebrobasilar stent placement on cerebral perfusion and cognition. AJNR Am J Neuroradiol. 2005 Aug;26(7):1772-80. PubMed PMID: 16091529.
8. Grunwald IQ, Supprian T, Politi M, Struffert T, Falkai P, Krick C, Backens M, Reith W. Cognitive changes after carotid artery stenting. Neuroradiology. 2006 May;48(5):319-23. Epub 2006 Apr 7. PubMed PMID: 16703361.
9. Carta MG, Lecca ME, Saba L, et al. Patients with carotid atherosclerosis who underwent or did not undergo carotid endarterectomy: outcome on mood, cognition and quality of life. BMC Psychiatry. 2015;15:277. doi:10.1186/s12888-015-0663-y.
10. Wapp M, Everts R, Burren Y, Kellner-Weldon F, El-Koussy M, Wiest R, Federspiel A, Michel P, Schroth G. Cognitive improvement in patients with carotid stenosis is independent of treatment type. Swiss Med Wkly. 2015 Dec 23;145:w14226. doi: 10.4414/smw.2015.14226. eCollection 2015. PubMed PMID: 26700596.
11. Buchbinder BR. Functional magnetic resonance imaging. Handb Clin Neurol. 2016;135:61-92. doi: 10.1016/B978-0-444-53485-9.00004-0. Review. PubMed PMID: 27432660.
12. Mori S, Zhang J. Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron. 2006 Sep 7;51(5):527-39. Review. PubMed PMID: 16950152.
13. Lin C-J, Tu P-C, Chern C-M, et al. Connectivity Features for Identifying Cognitive Impairment in Presymptomatic Carotid Stenosis. Yu C, ed. PLoS ONE. 2014;9(1):e85441. doi:10.1371/journal.pone.0085441.
14. Chang TY, Huang KL, Ho MY, Ho PS, Chang CH, Liu CH, Chang YJ, Wong HF, Hsieh IC, Lee TH, Liu HL. Graph theoretical analysis of functional networks and its relationship to cognitive decline in patients with carotid stenosis. J Cereb Blood Flow Metab. 2016 Apr;36(4):808-18. doi: 10.1177/0271678X15608390. Epub 2015 Oct 1. PubMed PMID: 26661184; PubMed Central PMCID: PMC4820004.
15. Avirame K, Lesemann A, List J, Witte AV, Schreiber SJ, Flöel A. Cerebral autoregulation and brain networks in occlusive processes of the internal carotid artery. Journal of Cerebral Blood Flow & Metabolism. 2015;35(2):240-247. doi:10.1038/jcbfm.2014.190.
16. Wang T, Xiao F, Wu G, et al. Impairments in Brain Perfusion, Metabolites, Functional Connectivity, and Cognition in Severe Asymptomatic Carotid Stenosis Patients: An Integrated MRI Study. Neural Plasticity. 2017;2017:8738714. doi:10.1155/2017/8738714.
17. Cheng HL, Lin CJ, Soong BW, Wang PN, Chang FC, Wu YT, Chou KH, Lin CP, Tu PC, Lee IH. Impairments in cognitive function and brain connectivity in severe asymptomatic carotid stenosis. Stroke. 2012 Oct;43(10):2567-73. Epub 2012 Aug 30. PubMed PMID: 22935402.
18. Wang T, Sun D, Liu Y, et al. The Impact of Carotid Artery Stenting on Cerebral Perfusion, Functional Connectivity, and Cognition in Severe Asymptomatic Carotid Stenosis Patients. Frontiers in Neurology. 2017;8:403. doi:10.3389/fneur.2017.00403.
19. Lin CJ, Chang FC, Chou KH, Tu PC, Lee YH, Lin CP, Wang PN, Lee IH. Intervention versus Aggressive Medical Therapy for Cognition in Severe Asymptomatic Carotid Stenosis. AJNR Am J Neuroradiol. 2016 Apr 28. [Epub ahead of print] PubMed PMID: 27127004.
20. Schaaf M, Mommertz G, Ludolph A, Geibprasert S, Mühlenbruch G, Das M, Krings T. Functional MR imaging in patients with carotid artery stenosis before and after revascularization. AJNR Am J Neuroradiol. 2010 Nov;31(10):1791-8. doi: 10.3174/ajnr.A2219. Epub 2010 Aug 26. PubMed PMID: 20801766.
21. Yeh FC, Badre D, Verstynen T. Connectometry: A statistical approach harnessing the analytical potential of the local connectome. Neuroimage. 2016 Jan 15;125:162-171. doi: 10.1016/j.neuroimage.2015.10.053. Epub 2015 Oct 21. PubMed PMID: 26499808.

22. Yeh FC, Tang PF, Tseng WY. Diffusion MRI connectometry automatically reveals affected fiber pathways in individuals with chronic stroke. Neuroimage Clin. 2013 Jun 29;2:912-21. doi: 10.1016/j.nicl.2013.06.014. eCollection 2013. PubMed PMID: 24179842; PubMed Central PMCID: PMC3777702.
23. Yeh FC, Tseng WY. NTU-90: a high angular resolution brain atlas constructed by q-space diffeomorphic reconstruction. Neuroimage. 2011 Sep 1;58(1):91-9. doi: 10.1016/j.neuroimage.2011.06.021. Epub 2011 Jun 16. PubMed PMID: 21704171.
24. Sobhani S, Rahmani F, Aarabi MH, Sadr AV. Exploring white matter microstructure and olfaction dysfunction in early parkinson disease: diffusion MRI reveals new insight. Brain Imaging Behav. 2017 Nov 13. doi: 10.1007/s11682-017-9781-0. [Epub ahead of print] PubMed PMID: 29134611.
25. Haghshomar M, Rahmani F, Hadi Aarabi M, Shahjouei S, Sobhani S, Rahmani M. White Matter Changes Correlates of Peripheral Neuroinflammation in Patients with Parkinson's Disease. Neuroscience. 2017 Nov 8. pii: S0306-4522(17)30789-3. doi: 10.1016/j.neuroscience.2017.10.050. [Epub ahead of print] PubMed PMID: 29126955.
26. Monnot C, Zhang X, Nikkhou-Aski S, Damberg P, Svenningsson P. Asymmetric dopaminergic degeneration and levodopa alter functional corticostriatal connectivity bilaterally in experimental parkinsonism. Exp Neurol. 2017 Jun;292:11-20. doi: 10.1016/j.expneurol.2017.02.014. Epub 2017 Feb 20. PubMed PMID: 28223037; PubMed Central PMCID: PMC5405850.
27. Rahmani F, Aarabi MH. Does apolipoprotein A1 predict microstructural changes in subgenual cingulum in early Parkinson? J Neurol. 2017 Apr;264(4):684-693. doi: 10.1007/s00415-017-8403-5. Epub 2017 Feb 6. PubMed PMID: 28168521.
28. Ansari M, Rahmani F, Dolatshahi M, Pooyan A, Aarabi MH. Brain pathway differences between Parkinson's disease patients with and without REM sleep behavior disorder. Sleep Breath. 2017 Mar;21(1):155-161. doi: 10.1007/s11325-016-1435-8. Epub 2016 Nov 16. PubMed PMID: 27853964.
29. Wen MC, Heng HS, Ng SY, Tan LC, Chan LL, Tan EK. White matter microstructural characteristics in newly diagnosed Parkinson's disease: An unbiased whole-brain study. Sci Rep. 2016 Oct 20;6:35601. doi: 10.1038/srep35601. PubMed PMID: 27762307; PubMed Central PMCID: PMC5071859.
30. Rahmani F, Ansari M, Pooyan A, Mirbagheri MM, Aarabi MH. Differences in white matter microstructure between Parkinson's disease patients with and without REM sleep behavior disorder. Conf Proc IEEE Eng Med Biol Soc. 2016 Aug;2016:1124-1126. doi: 10.1109/EMBC.2016.7590901. PubMed PMID: 28268523.
31. Delaparte L, Yeh FC, Adams P, Malchow A, Trivedi MH, Oquendo MA, Deckersbach T, Ogden T, Pizzagalli DA, Fava M, Cooper C, McInnis M, Kurian BT, Weissman MM, McGrath PJ, Klein DN, Parsey RV, DeLorenzo C. A comparison of structural connectivity in anxious depression versus non-anxious depression. J Psychiatr Res. 2017 Jun;89:38-47. doi: 10.1016/j.jpsychires.2017.01.012. Epub 2017 Jan 24. PubMed PMID: 28157545; PubMed Central PMCID: PMC5374003.
32. Olvet DM, Delaparte L, Yeh FC, DeLorenzo C, McGrath PJ, Weissman MM, Adams P, Fava M, Deckersbach T, McInnis MG, Carmody TJ, Cooper CM, Kurian BT, Lu H, Toups MS, Trivedi MH, Parsey RV. A COMPREHENSIVE EXAMINATION OF WHITE MATTER TRACTS AND CONNECTOMETRY IN MAJOR DEPRESSIVE DISORDER. Depress Anxiety. 2016 Jan;33(1):56-65. doi: 10.1002/da.22445. Epub 2015 Oct 19. PubMed PMID: 26477532; PubMed Central PMCID: PMC4701622.
33. Romascano D, Meskaldji DE, Bonnier G, Simioni S, Rotzinger D, Lin YC, Menegaz G, Roche A, Schluep M, Pasquier RD, Richiardi J, Van De Ville D, Daducci A, Sumpf T, Fraham J, Thiran JP, Krueger G, Granziera C. Multicontrast connectometry: a new tool to assess cerebellum alterations in early relapsing-remitting multiple sclerosis. Hum Brain Mapp. 2015 Apr;36(4):1609-19. doi: 10.1002/hbm.22698. Epub 2014 Nov 24. PubMed PMID: 25421928.
34. Abhinav K, Yeh FC, El-Dokla A, Ferrando LM, Chang YF, Lacomis D, Friedlander RM, Fernandez-Miranda JC. Use of diffusion spectrum imaging in preliminary longitudinal evaluation of amyotrophic lateral sclerosis: development of an imaging biomarker. Front Hum Neurosci. 2014 Apr 29;8:270. doi: 10.3389/fnhum.2014.00270. eCollection 2014. PubMed PMID: 24808852; PubMed Central PMCID: PMC4010737.
35. Kakisis JD, Avgerinos ED, Antonopoulos CN, Giannakopoulos TG, Moulakakis K, Liapis CD. The European Society for Vascular Surgery guidelines for carotid intervention: an updated independent assessment and literature review. Eur J Vasc Endovasc Surg. 2012 Sep;44(3):238-43. doi: 10.1016/j.ejvs.2012.04.015. Epub 2012 Jun 1. Review. PubMed PMID: 22658616.
36. Julious, S. A. (2005), Sample size of 12 per group rule of thumb for a pilot study. Pharmaceut. Statist., 4: 287–291. doi:10.1002/pst.185
37. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke. 1988 May;19(5):604-7. PubMed PMID: 3363593.
38. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975 Nov;12(3):189-98. PubMed PMID: 1202204.
39. Magni E, Binetti G, Bianchetti A, Rozzini R, Trabucchi M. Mini-Mental State Examination: a normative study in Italian elderly population. Eur J Neurol. 1996 May;3(3):198-202. doi: 10.1111/j.1468-1331.1996.tb00423.x. PubMed PMID: 21284770.
40. Yamauchi H, Fukuyama H, Nagahama Y, Shiozaki T, Nishizawa S, Konishi J, Shio H, Kimura J. Brain arteriolosclerosis and hemodynamic disturbance may induce leukoaraiosis. Neurology. 1999 Nov 10;53(8):1833-8. PubMed PMID: 10563635.
41. Yeh FC, Wedeen VJ, Tseng WY. Generalized q-sampling imaging. IEEE Trans Med Imaging. 2010 Sep;29(9):1626-35. doi: 10.1109/TMI.2010.2045126. Epub 2010 Mar 18. PubMed PMID: 20304721.
42. Yeh FC, Liu L, Hitchens TK, Wu YL. Mapping immune cell infiltration using restricted diffusion MRI. Magn Reson Med. 2017 Feb;77(2):603-612. doi: 10.1002/mrm.26143. Epub 2016 Feb 4. PubMed PMID: 26843524.
43. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. Hum Brain Mapp. 2002 Jan;15(1):1-25. Pubmed PMID: 11747097.
44. Yeh F-C, Verstynen TD, Wang Y, Fernández-Miranda JC, Tseng W-YI. Deterministic Diffusion Fiber Tracking Improved by Quantitative Anisotropy. Zhan W, ed. PLoS ONE. 2013;8(11):e80713. doi:10.1371/journal.pone.0080713.
45. Zuniga MC, Tran TB, Baughman BD, Raghuraman G, Hitchner E, Rosen A, Zhou W. A Prospective Evaluation of Systemic

Biomarkers and Cognitive Function Associated with Carotid Revascularization. Ann Surg. 2016 Oct;264(4):659-65. doi: 10.1097/SLA.0000000000001853. PubMed PMID: 27433899; PubMed Central PMCID: PMC5348259.

46. Chang EH, Argyelan M, Aggarwal M, Chandon TS, Karlsgodt KH, Mori S, Malhotra AK. The role of myelination in measures of white matter integrity: Combination of diffusion tensor imaging and two-photon microscopy of CLARITY intact brains. Neuroimage. 2017 Feb 15;147:253-261. doi: 10.1016/j.neuroimage.2016.11.068. Epub 2016 Dec 13. PubMed PMID: 27986605; PubMed Central PMCID: PMC5560594.
47. Yeh F-C, Vettel JM, Singh A, et al. Quantifying Differences and Similarities in Whole-Brain White Matter Architecture Using Local Connectome Fingerprints. Diedrichsen J, ed. PLoS Computational Biology. 2016;12(11):e1005203. doi:10.1371/journal.pcbi.1005203.
48. van der Knaap LJ, van der Ham IJ. How does the corpus callosum mediate interhemispheric transfer? A review. Behav Brain Res. 2011 Sep 30;223(1):211-21. doi: 10.1016/j.bbr.2011.04.018. Epub 2011 Apr 21. Review. PubMed PMID: 21530590.
49. Renard D, Castelnovo G, Campello C, Bouly S, Le Floch A, Thouvenot E, Waconge A, Taieb G. An MRI review of acquired corpus callosum lesions. J Neurol Neurosurg Psychiatry. 2014 Sep;85(9):1041-8. doi: 10.1136/jnnp-2013-307072. Epub 2014 Feb 21. Review. PubMed PMID: 24563521.
50. Buckner RL. The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. Neuron. 2013 Oct 30;80(3):807-15. doi: 10.1016/j.neuron.2013.10.044. Review. PubMed PMID: 24183029.
51. Sullivan EV. Cognitive Functions of the Cerebellum. Neuropsychology review. 2010;20(3):227-228. doi:10.1007/s11065-010-9144-8.
52. Balsters JH, Cussans E, Diedrichsen J, Phillips KA, Preuss TM, Rilling JK, Ramnani N. Evolution of the cerebellar cortex: the selective expansion of prefrontal-projecting cerebellar lobules. Neuroimage. 2010 Feb 1;49(3):2045-52. doi: 10.1016/j.neuroimage.2009.10.045. Epub 2009 Oct 24. PubMed PMID: 19857577.
53. Kelly RM, Strick PL. Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. J Neurosci. 2003 Sep 10;23(23):8432-44. PubMed PMID: 12968006.
54. Gellersen HM, Guo CC, O'Callaghan C, Tan RH, Sami S, Hornberger M. Cerebellar atrophy in neurodegeneration-a meta-analysis. J Neurol Neurosurg Psychiatry. 2017 Sep;88(9):780-788. doi: 10.1136/jnnp-2017-315607. Epub 2017 May 13. Review. PubMed PMID: 28501823.

Table Legend

Table 1: Demographic data.

Table 2: individual mean sdf values of corpus callosum, right and left cerebellum.

Figures Legends

Figure 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.

Figure 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.

Figure 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).

Figure 4: p-FDR trend (vertical axis) in relation to fiber length (horizontal axis) for T-Score threshold = 3.