Theme 8 Imaging

IMG-01 SPG11-related motor neuron disease is a mixed neurodevelopmental white matter and neurodegenerative grey matter condition

I Faber¹, A Martinez¹, T De Rezende¹, M Martins¹, CR Martins Jr¹, C Lourenço², W Marques Jr², C Montecchiani³, A Orlacchio³, JL Pedroso³, O Barsottini⁴, I Lopes-Cendes¹, M França Jr¹

¹University of Campinas (UNICAMP), CAMPINAS, Brazil, ²University of São Paulo (USP-RP), Ribeirão Preto, Brazil, ³Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Santa Lucia, Rome, Italy, ⁴Federal University of São Paulo (UNIFESP), São Paulo, Brazil

Email address for correspondence: ingridfaber81@gmail.com

Keywords: neuroimaging, dementia, SPG11

Background: Mutations in SPG11 gene account for most of the Autosomal Recessive Hereditary Spastic Paraplegias and is also the leading cause of juvenile ALS (1). Neuroimaging techniques have proven to be a powerful tool in ALS and other motor neuron disorders, providing a better understanding of the disease mechanisms and progression. Nonetheless, no large studies have been devoted to this rare disorder.

Objectives: To investigate the extent of neurodegeneration associated with SPG11-MND, its clinical correlates and natural history.

Methods: 25 SPG11-MND patients were subject to a comprehensive neurological assessment; disease severity was quantified through the Spastic Paraplegia Rating Scale (SPRS) and cognition was measured through the Addenbrooke’s Cognitive Examination Revised (ACE-R). All subjects underwent MRI in a 3T scanner. T1 weighted images were used to assess cortical thickness through the Freesurfer software and deep grey matter (GM) volume through the MultiAtlas tool. White matter (WM) microstructural damage was assessed using raw diffusion tensor imaging (DTI) that images that were subject to tract-based spatial statistics and DTI MultiAtlas. Spinal cord morphometry was analyzed with the Spineseg software. MRI analysis included comparison with a group of healthy sex and age-matched controls (n = 25), correlation with clinical outcomes, as well as time-related measures (age and disease duration). p-values <0.05 (Dunn-Sidak corrected) were considered significant.

Results: Mean age and disease duration were 29 (range = 18–49) and 13.2 (range = 0–30) years, respectively. Progressive spastic paraplegia was the main symptom in all cases and 16 (64%) were wheelchair-bound at evaluation. The disease’s annual rate of progression across one year was 3.65 points in the SPRS (range = 0–52). ACE-R revealed dementia in 21 (84%) patients with predominant frontotemporal dysfunction. GM analysis unfolded diffuse basal ganglia and thalamic atrophy and a more restricted pattern of cortical thinning, including motor, limbic and parietal regions. Motor cortices and basal ganglia atrophy were associated with motor handicap, while associative cortex and thalamic damage correlated with poor cognitive performance. WM integrity loss was diffuse and, at the fornices and corpus callosum, such damage correlated with poor cognition. Spinal cord atrophy correlated with motor handicap. Paracentral gyri, basal ganglia and spinal cord metrics correlated inversely with age and disease duration, while WM integrity did not display significant correlation in regard to age or disease duration.

Discussion and conclusion: SPG11-MND causes a distinctive pattern of developmental WM and degenerative GM disease. Frontotemporal subcortical damage underlies SPG11-related dementia.

Acknowledgments: This work was supported by the São Paulo Research Foundation, Fapesp, the Brazilian National Council for Scientific and Technological Development, CNPq and the Italian Ministero della Salute.

Reference

1. Kara E, Tuxxi A, Manzoni C, et al. Brain. 2016;139:1904–18

DOI: 10.1080/21678421.2017.1374605/001

IMG-02 Phenotype-specific imaging signatures along the ALS-FTD spectrum

E Finegan¹, T Omer¹, S Hutchinson², M Doherty³, A Vajda¹, R McLaughlin³, N Pender¹, O Hardiman¹, P Bede¹

¹Quantitative Neuroimaging Group, Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, ²Department of Neurology, St James's Hospital, Dublin, Ireland, ³Population Genetics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland
**Background:** Frontotemporal dementia is associated with considerable clinical, genetic and pathological heterogeneity.

**Objective:** The objective of this study is to characterise the imaging signatures of the main FTD phenotypes along the ALS-FTD spectrum.

**Methods:** A total of 100 participants underwent comprehensive multimodal neuroimaging, genetic testing and neuropsychological evaluation. Seven patients with behavioural variant FTD (bvFTD), 11 patients with non-fluent-variant primary progressive aphasia (nfvPPA), two patients with semantic-variant primary progressive aphasia (svPPA), 10 patients with amyotrophic lateral sclerosis and FTD carrying the C9orf72 hexanucleotide repeat (C9 + ALS-FTD), 10 patients with ALS-FTD without hexanucleotide repeats (C9-ALS-FTD), 20 ALS patients without behavioural or cognitive deficits (ALSnci) and 40 healthy controls (HC) were included in a prospective quantitative neuroimaging study.

**Results:** Phenotype-specific spatial patterns of pathology were identified along the ALS-FTD spectrum, highlighting a strikingly focal distribution of disease-burden as opposed to global atrophy. Significant motor cortex and corticospinal tract degeneration was identified in both bvFTD and nfvPPA patients. ALS-FTD patients exhibited widespread extra-motor pathology and significant precentral gyrus atrophy compared to ALSnci patients. ROI analyses confirmed focal grey matter alterations in Broca's and Wernicke's area in language variant FTD cohorts.

**Conclusion:** Our findings confirm that the clinical manifestations of FTD are underpinned by phenotype-specific patterns of white and grey matter degeneration.

**Acknowledgments:** We gratefully acknowledge the kindness and generosity of our patients and their caregivers. This work was supported by the Irish Institute of Clinical Neuroscience (IICN), Novartis Ireland Research Grant, the Iris O’Brien Foundation, the Perrigo Clinician-Scientist Research Fellowship, the Health Research Board and the Research Motor Neuron (RMN-Ireland) foundation.

DOI: 10.1080/21678421.2017.1374605/002

**IMG-03 Virtual brain biopsies in ALS: a diagnostic framework based on in-vivo pathological patterns**

P Bede, P Iyer, E Finegan, T Omer, O Hardiman

Trinity College Dublin, Dublin, Ireland

**Keywords:** neuroimaging, cognition, frontotemporal dementia

**Background:** Diagnostic uncertainty in ALS has serious management implications and delays recruitment into clinical trials. Emerging evidence of presymptomatic disease-burden provides the rationale to develop diagnostic applications based on the evaluation of in-vivo pathological patterns early in the disease. After years of descriptive MRI studies in ALS, a number of studies have now emerged outlining diagnostic and prognostic applications based on pattern recognition.

**Objectives:** To outline and test a diagnostic classification approach based on an array of complementary imaging metrics in key disease-associated anatomical structures.

**Methods:** All participants of this prospective biomarker study provided informed consent in accordance with the Medical Ethics Approval of the project (Beaumont Hospital, Dublin, Ireland). Data from 75 ALS patients and 75 healthy controls were randomly allocated in a 'training' and 'validation' cohort. Spatial masks were created for anatomical foci which best discriminate patients from controls in the 'training sample'. In a virtual 'brain biopsy', data was then retrieved from these key disease-associated brain regions. White matter diffusivity indices, grey matter T1-signal intensity values and basal ganglia volumes were evaluated as predictor variables in a canonical discriminant function.

**Results:** Following predictor variable selection, the canonical discriminant function reached an eigenvalue of 0.871, a canonical correlation value of 0.682, Wilks lambda of 0.534, Chi-square of 62.983 and a significance of less than 0.001. The model showed an overall classification sensitivity of 89.1% and specificity of 85.5% in the 'training' group. In the 'testing sample', both sensitivity and specificity reached 90%.

**Discussion:** This study evaluates disease-associated imaging measures in a diagnostic application. From a biomarker perspective, the meaningful interpretation of data from single individuals is paramount for the development of viable clinical applications. Although larger samples will be required for robust validation, the study confirms the potential of multimodal quantitative imaging in clinical applications. The concept of region-of-interest (ROI)-based, spatial reference system guided 'data biopsies' is also applicable to longitudinal analyses and potentially for the development of monitoring markers.

**Acknowledgments:** We gratefully acknowledge the kindness and generosity of our patients and their caregivers for participating in this study.

DOI: 10.1080/21678421.2017.1374605/003
IMG-04 Imaging and physiology markers of disease progression in C9orf72 mutation carriers

MK Floeter, LE Danielian, D Bageac, T Lehky, M Offit, MG Clark, R Smallwood, T Wu

National Institute of Neurological Disorders and Stroke, NIH, Bethesda, USA

Email address for correspondence: floeterm@ninds.nih.gov

Keywords: C9orf72, DTI, TMS

Background: Repeat expansion mutations in C9orf72 may account for 10% of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in the US. Patients can have a mixture of ALS and FTD findings. To select appropriate outcome measures for future clinical trials targeting the C9orf72 mutation, it is important to establish the natural history of disease progression for different clinical phenotypes. Another goal is to assess changes in candidate imaging and physiology biomarkers over the duration of a typical clinical trial.

Methods: Prospective study of 39 adult C9orf72 mutation carriers (17 with ALS, 7 ALS-FTD, 3 FTD and 11 asymptomatic carriers) at enrolment (baseline), 6- and 18-month follow-up. ALSFRS-R, King’s staging (modified), Fluency and Frontal Behavioural Inventory (FBI) used to document severity of motor and cognitive impairment at each time-point. Tested outcomes included: cortical excitability thresholds with transcranial magnetic stimulation (TMS) recording from thenar muscles, electroimpedance myography phase at 50 Hz (EIM); 3T T1 MRI for measures of cortical thickness and volumes (FreeSurfer) and diffusion tensor imaging (DTI) to obtain measures of fractional anisotropy (FA) and mean diffusivity (MD) of white matter tracts (Tortoise, FSL TBSS, MRI Studio).

Results: Cortical thresholds were lower in C9 + ALS/ALS-FTD patients at King’s stage 1 than at later stages and in asymptomatic carriers. EIM declined over 18 months in most C9 + ALS/ALS-FTD patients. Score on ALSFRS-R gross motor items correlated with EIM of four leg muscles. Cortical and thalamic atrophy progressed over 6 months; ventricular volume correlated with Fluency and FBI scores. Regions of white matter with reduced FA and increased MD expanded over 6 months in symptomatic patients. Altered FA and MD of frontal regions correlated with Fluency and FBI scores. Alterations of the right corticospinal tract correlated with ALSFRS-R. Whole-tract diffusion measures of corticospinal tracts, *uncinat e fasciculi* and segments of corpus callosum differ between clinical diagnoses, but remain stable in individuals over follow-up through 18 months.

Conclusion: In this population of C9orf72 mutation carriers with a mixture of motor and cognitive symptoms, clinical scales and findings of imaging and physiology studies were generally in agreement. ALSFRS-R and King’s ALS staging were correlated with physiology of upper and lower motor neurons and diffusion imaging properties of corticospinal tract white matter. Fluency and FBI scores were correlated with global atrophy and reduced integrity of frontal white matter. Over time, white matter changes extended more posteriorly and from deeper to more superficial white matter. Although group measures showed progression over 6- or 18-month follow-up, none of the biomarkers were sensitive in isolation to detect progression in individual patients. Future analyses will explore incorporation of multiple measures into an index of progression.

Acknowledgments: Supported by the Intramural Program National Institute Neurological Disorders and Stroke, NIH. Z01 NS003146.

DOI: 10.1080/21678421.2017.1374605/004

IMG-05 MRI and DTI predictors of reduced survival across the ALS-FTSD continuum

PM Ferraro1,2, L Massimo1, K Placek1, C Quinn3, F Agosta2, L Elman3, L McCluskey3, DJ Irwin1, M Filippi2, M Grossman1, CT McMillan1

1Department of Neurology, Penn Frontotemporal Degeneration Center, University of Pennsylvania, Philadelphia, USA,
2Neuroimaging Research Unit, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy, 3Penn Comprehensive ALS Center, University of Pennsylvania, Philadelphia, USA

Email address for correspondence: ferrarop@mail.med.upenn.edu

Keywords: survival, neuroimaging, frontotemporal dementia

Background: Amyotrophic lateral sclerosis (ALS) – frontotemporal degeneration spectrum disorders (FTSD) have distributed grey matter (GM) and white matter (WM) atrophy and heterogeneous rates of survival. However, little is known about how GM and WM atrophy relates to survival.

Objective: To investigate whether GM Magnetic Resonance Imaging (MRI) and WM Diffusion Tensor Imaging (DTI) provide potential prognostic markers of survival in ALS-FTSD.

Methods: We evaluated ALS patients with a pure motor syndrome (ALS-motor, * n = 19*), ALS patients with FTD (ALS-FTD, * n = 24*) and behavioral variant FTD patients (bvFTD, * n = 14*) with either a neuropathological diagnosis and/or a known genetic mutation associated with FTLD-TDP pathology using MRI, DTI and survival data (estimated as the time from MRI to death). We used Advanced Normalization Tools (ANTs) to compute cortical thickness (CT) of GM and fractional anisotropy (FA) of WM. Univariate regression models were used to test the
relationship between reduced survival and MRI variables, adjusting for disease duration ($p<0.05$ unc).

Results: The longest survival was observed in bvFTD patients ($3.85 \pm 1.55$), followed by ALS-motor ($2.05 \pm 1.23$) and ALS-FTD patients ($1.54 \pm 1.32$). In bvFTD patients, reduced survival was related to CT reductions encompassing the amygdala, orbitofrontal and temporal cortices and only modest temporal WM alterations. In ALS-motor patients, the best predictors of reduced survival were CT reductions in primary motor regions and temporal areas and damage of the corticospinal tract (CST) and corpus callosum (CC). In ALS-FTD patients, reduced survival was predicted by CT decreases in primary motor regions, temporal and parietal cortices, as well as damage of the CST, CC and temporal WM tracts.

Discussion and conclusion: Our results suggest expanding brain network degeneration to be a key determinant of progressive disease course in ALS-FTSD. Accordingly, GM alterations extending beyond the motor system were the best predictors of reduced survival in both ALS and ALS-FTD phenotypes, while confined GM changes in bvFTD were associated with a more benign disease course. Notably, pronounced WM damage associated with reduced survival was mainly observed in ALS and ALS-FTD phenotypes, suggesting WM fibers as important pathways in damage propagation across these conditions. In conclusion, we suggest that structural MRI and DTI may be able to detect differences in vulnerability of neural networks to damage. We further suggest that MRI and DTI may provide feasible prognostic markers for mortality in the ALS-FTD spectrum disorders.

Acknowledgments: This work was supported by NIH (AG043503, AG17856) and Dana Foundation.

Keyword: neuroimaging, cognition, C9orf72

Background: The association of amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD) is well recognised. Since the discovery of the hexanucleotide repeat expansion in C9orf72 in 2011, the neuroimaging profile of patients carrying the repeat has been linked to orbitofrontal and temporal pathology. It is clear, however, that the C9orf72 genotype does not account for all ALS-FTD patients and relatively little is known of the imaging profile of ALS-FTD patients who don’t carry the hexanucleotide expansion.

Objectives: The objective of this study is to comprehensively characterise and compare the neuroimaging profiles of C9orf72 positive and negative patients.

Methods: 10 patients with ALS-FTD carrying the C9orf72 hexanucleotide repeat (C9+ ALS-FTD) and 10 patients with ALS-FTD without the C9orf72 repeat (C9− ALS-FTD) were included in a prospective quantitative neuroimaging study using 3T MRI. 20 cognitively normal ALS patients were also included for comparison. Cortical grey matter morphometry analyses were performed using both a whole-brain and region-of-interest approach. Multiparametric diffusion tensor imaging (DTI) analyses were carried out with affected regions explored for axial diffusivity (AD), mean diffusivity (MD), radial diffusivity (RD) and fractional anisotropy (FA), with statistical significance defined at $p<0.05$.

Results: Compared with cognitively normal ALS group, grey matter abnormalities were more significant in the C9− ALS-FTD group than in the C9+ ALS-FTD group. Furthermore, ALS-FTD patients showed extensive extra-motor white-matter pathology in comparison to the cognitively normal ALS group. FA and RD changes in orbitofrontal and pre-central regions were more pronounced in the C9− ALS-FTD group than in the C9+ ALS-FTD group. AD changes were less extensive overall than other indices although, once again, changes were more apparent in the C9− ALS-FTD group.

Conclusion: This study characterises, in-vivo, the widespread extra-motor changes in C9orf72 negative ALS-FTD in our study are more extensive than in those carrying the hexanucleotide repeat in C9orf72.

Acknowledgments: We gratefully acknowledge the kindness and generosity of our patients and their caregivers. This work was supported by the Irish Institute of Clinical Neuroscience (IICN), Novartis Ireland Research Grant, the Iris O’Brien Foundation, the Perrigo Clinician-Scientist Research Fellowship, the Health Research Board and the Research Motor Neuron (RMN-Ireland) foundation.

Keyword: neuroimaging, cognition, C9orf72

IMG-06 Beyond C9ORF72: neuroradiological characterisation of hexanucleotide repeat negative ALS-FTD patients

E Finegan1, T Omer1, S Hutchinson2, M Doherty3, A Vajda1, R McLaughlin3, N Pender1, O Hardiman1, P Bede1

1Quantitative Neuroimaging Group, Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland, 2Department of Neurology, St James’s Hospital, Dublin, Ireland, 3Population Genetics Laboratory, Smurfit Institute of Genetics, Trinity College Dublin, Dublin, Ireland

Email address for correspondence: finegane@tcd.ie

Keyword: neuroimaging, cognition, C9orf72
**IMG-07 Structural and functional Papez circuit integrity in amyotrophic lateral sclerosis**

AP Arantes Bueno\(^1\), WHL Pinaya\(^1\), LM Moura\(^1\), ML Bertoux\(^2\), R Radakovic\(^2\), M Kiernan\(^3\), AL Teixiera\(^4\), L Cruz de Souza\(^4\), M Hornberger\(^2\), J Ricardo Sato\(^1\)

\(^1\)Universidade Federal do ABC, Santo André, Brazil, \(^2\)University of East Anglia, Norwich, United Kingdom, \(^3\)Brain and Mind Institute, Sydney University, Sydney, Australia, \(^4\)Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

**Email address for correspondence:** apbueno74@gmail.com

**Keywords:** multimodal MRI, Papez circuit, episodic memory

**Background:** Cognitive impairment in amyotrophic lateral sclerosis (ALS) is heterogeneous, but now recognized as a feature in non-demented patients and no longer exclusively attributed to executive dysfunction. However, despite common reports of temporal lobe changes and memory deficits in ALS, episodic memory has been less explored.

**Objectives and methods:** The current study explores: 1) how the hippocampus and associated Papez circuit structures are structurally (cortical volume and thickness, voxel-based morphometry, diffusion tensor imaging) and functionally (resting state functional connectivity) affected in ALS patients (n = 20) compared with healthy controls (HC, n = 15) and 2) whether these changes correlate with a commonly used clinical measure of episodic memory (ACE-R).

**Results:** ALS patients and HC did not differ on age (p = 0.29). Mean education of ALS patients in years was 12.5 ± 3.56 (SD). Spearman correlation coefficient did not show a significant correlation between disease duration (2.61 ± 2.17) and brain damage. Our findings show reduced gray matter (GM) in the left hippocampus (p = 0.03), left entorhinal cortex (p = 0.01) and right posterior cingulate (p = 0.02) of ALS patients. We found decreased white matter (WM) fractional anisotropy (p = 0.04) and increased mean diffusivity (p = 0.02) in the left cingulum bundle (hippocampal part) of ALS patients. Interestingly, mammillary bodies and fornix were preserved. Finally, we report a decrease in functional connectivity in ALS patients in bilateral hippocampus, bilateral posterior and anterior parahippocampal gyrus and posterior cingulate (all p-FDR ≤ 0.04 at ROI level). Spearman correlations showed GM atrophy and mean diffusivity correlated significantly with memory measures (all p ≤ 0.04).

**Discussion and conclusion:** The results reveal that ALS patients showed significant GM and WM structural changes, but also more widespread significant functional connectivity abnormalities across the whole Papez circuit. The decreased functional connectivity found in the Papez network may indicate these changes could be used to assess risk or assist early detection or development of memory symptoms in ALS patients, even before structural changes are established. Future studies investigating the longitudinal changes of Papez circuit integrity are needed to explore this further.

DOI: 10.1080/21678421.2017.1374605/007

**IMG-08 Neuroimaging changes in the first 5 years of symptoms in patients with primary lateral sclerosis**

M Clark, C Huang, D Bageac, L Danielian, R Smallwood, MK Floeter

National Institute of Neurological Disorders and Stroke, NIH, Bethesda, USA

**Email address for correspondence:** floeterm@ninds.nih.gov

**Keywords:** primary lateral sclerosis, functional connectivity, multimodal MRI

**Background:** Primary lateral sclerosis (PLS) is a disorder of unknown etiology characterized by insidious onset and progression of spasticity without clinical lower motor neuron signs. Because ALS can initially present with only upper motor neuron signs, a clinical diagnosis of PLS is only made after pure upper motor symptoms have been present for 4 years or longer. Imaging findings in patients who meet clinical criteria for PLS are likely to represent late findings of degeneration. No studies to date have assessed imaging changes that occur early in the course of PLS. To explore early imaging changes in PLS, we prospectively scanned patients with progressive spasticity for five years or less and followed them clinically to retain those that met criteria for clinically pure PLS. Imaging findings in these ‘pre-PLS’ patients were compared to ‘established’ PLS patients and healthy age-matched controls. We hypothesized that functional imaging changes would precede structural changes.

**Methods:** Participants were scanned between 2012–2016; clinical follow-up through to 2017. GE 3T MRI. Sequences included high-resolution T1 weighted sequence for measures of cortical thickness and volumes (FreeSurfer); resting state functional MRI (rs-fMRI) using VOIs from FreeSurfer Desikan-Killiany atlas, evaluated in FSL and with custom MATLAB scripts and the Brain Connectivity Toolbox; diffusion tensor imaging (DTI) for fractional anisotropy (FA) and mean diffusivity (MD) of white matter tracts (Tortoise, FSL).

**Results:** 19 patients with spasticity <5 years were scanned; 13 patients (symptom duration 3.2 ± 1.3 years) later met criteria for PLS. Eighteen established PLS patients (13 ± 7 year symptom duration) and 23 controls. No age or gender differences. Edge-wise analysis of resting state functional connectivity networks showed that ‘pre-PLS’ patients had stronger connectivity between frontal, temporal, precuneus and isthmus of the cingulate regions compared to controls. Established PLS patients had reduced some of the same regions. Minimal cortical thinning was seen in pre-PLS patients. Established PLS...
patients had focal thinning of the precentral and paracentral gyrus. The normal structural covariance pattern (correlated thickness) between cortical regions differed in pre-PLS and established PLS compared to controls.

Summary: Functional connectivity increased between default mode hubs and other cortical regions in the first five years in patients with progressive spasticity who later met clinical criteria for PLS. This could reflect reorganization of functional networks with declining of motor cortex output. Over time the motor cortex became thinner and functional connectivity among cortical regions declined. Work is in progress to examine connectivity with subcortical regions and the cerebellum and to combine diffusion, structural and functional imaging to assess the temporal relationship between functional and structural imaging changes.

DOI: 10.1080/21678421.2017.1374605/008

IMG-09 Functional connectivity changes associated with disease progression in ALS

K Loewe1, J Machts1,2, S Petri3, H-J Heinze1,4, S Vielhaber1,2, MA Schoenfeld1,4,5

1Otto-von-Guericke University, Magdeburg, Germany, 2German Centre for Neurodegenerative Diseases, Magdeburg, Germany, 3Hannover Medical School, Hannover, Germany, 4Leibniz Institute for Neurobiology, Magdeburg, Germany, 5Kliniken Schmieder, Heidelberg, Germany

Email address for correspondence: judith.machts@med.ovgu.de

Keywords: functional connectivity, graph analysis, disease progression

Background: The development of novel biomarkers based on MRI techniques is a major objective of recent neuroimaging research. In the future, they may complement established clinical end-points such as motor tests and the ALSFRS-R score. To assess changes related to disease progression, most previous imaging studies have employed structural MRI measures. However, disease-related changes need to be rather substantial to be reliably detected. Recent findings indicate that functional MRI measures, such as resting-state connectivity, may be sensitive enough to facilitate the assessment of more subtle changes occurring on a finer time scale.

Objectives: To assess changes in functional connectivity associated with disease progression in ALS.

Methods: 38 patients with ALS and 18 healthy controls underwent resting-state fMRI at 3T (TR = 2200 ms, isotropic voxel size = 3.5 × 3.5 × 3.5 mm³) at baseline and after an interval of 3–6 months. For each subject and session, a dense connectome was constructed by defining gray matter voxels as nodes and estimating internodal functional connectivity in terms of the Pearson correlation between the nodes’ associated time series. For each group, edge-level statistics were computed based on the relevant connectomes to assess potential differences in connectivity between first and second session.

Results: Between-group differences in connectivity were observed in the motor- and sensorimotor system, the frontal lobe and the occipito-temporal lobe. Specifically, ALS-related connectivity reductions were found in the pre- and post-central gyri, the middle frontal gyri and the hippocampi. After 3–6 months, the patients exhibited decreased functional connectivity in the bilateral pre- and post-central gyri as compared to baseline. Similar reductions were observed in the bilateral cingulate gyrus (anterior and posterior) and also in the frontal (inferior frontal gyri, middle frontal gyri, frontal poles) and temporal lobes (bilateral hippocampus, temporal poles). Additionally, reduced functional connectivity was observed in the cerebellum. There were no significant increases in functional connectivity over time.

Discussion: ALS progression is reflected in widespread reductions in functional connectivity. Large patterns of connectivity decreases in the pre- and post-central gyrus are indicative of the ongoing neurodegeneration within primary motor areas. In addition, the observed connectivity reductions in parts of the default mode network – the hippocampus, the posterior cingulate gyrus and the frontal gyrus – are well in line with the decline in cognitive functioning typically reported during ALS progression. The observed reductions in connectivity, especially in the frontal lobes, might reflect a failure to (fully) sustain compensational mechanisms as the structural lesions enlarge with ongoing neurodegeneration.

DOI: 10.1080/21678421.2017.1374605/009

IMG-10 Integration of progressive white matter structural and functional MRI changes in motor neuron disease

R Menke1,2, G Douaud1,2, K Talbot2, M Turner1,2

1Wellcome Centre for Integrative Neuroimaging, Oxford, United Kingdom, 2Nuffield Department of Clinical Neurosciences, Oxford, United Kingdom

Email address for correspondence: ricarda.menke@ndcn.ox.ac.uk

Keywords: progression, MRI, connectivity

Background: We have previously described an integrated structural and functional connectivity approach which identified apparently dichotomous processes characterizing the MND cerebral network failure, in which there was increased functional connectivity within regions of decreased structural connectivity (1).
Objectives: The aim of this study was to further characterize the MND cerebral functional network failure in relation to structural changes using longitudinal data.

Methods: T1-weighted and resting state fMRI data was available for two time points in 31 patients (22 male, mean age at first scan = 61.4 ± 9.5 years, mean ALSFRS-R at first scan = 35.0 ± 5.4). Average intervals between subsequent time points were 6.6 ± 1.5 months. Grey matter segmentations were obtained using voxel-based morphometry (FSL-VBM) pre-processing pipelines. Five time point diffusion weighted data acquired over a period of two years available for a sub-set of 11 patients was used to define regions of consistent, long-term significant white matter decline via whole brain analyses of diffusion tensor imaging (DTI) metrics optimized for longitudinal analysed. Using probabilistic tractography in an independent group of age-matched, healthy controls, a grey matter connection network was defined based upon the regions of progressive white matter involvement demonstrated by consistently progressive DTI changes. This disease-specific grey matter network was then used as an input for a dual-regression analysis, to assess progressive changes in resting state functional connectivity (FC) directly associated with this network.

Results: Whole brain DTI analysis revealed consistently progressive decline of white matter structural integrity in the body of the corpus callosum, forceps minor, the anterior thalamic radiations, the right superior longitudi-nal fasciculus and the right inferior fronto-occipital fasciculus. Tractography from these areas led to a grey matter network including motor, premotor and supplementary motor cortices, pars opercularis and motor-related thalamic nuclei. Dual-regression analysis, however, did not reveal any significant progressive FC increases or decreases over time associated with this network.

Discussion and conclusion: While DTI analysis revealed progressive decline of structural integrity in white matter regions commonly reported to be affected in MND, such as the corpus callosum and the right superior longitudinal fasciculus, dual regression based on a grey matter network structurally connected with these white matter regions in controls did not reveal any specific progressive functional network failure in relation to white matter changes over an interval of 6 months. Ongoing analyses using a grey matter network defined by tractography in patients instead of healthy controls, as well as FC comparisons over a longer period of time, will shed more light on potential progressive white matter related FC changes. These are of particular importance, as fMRI has the ability to reflect disease mechanisms and potential therapeutic targets more directly.

Reference
1. Douaud G, Filippini N, Knight S, et al. Brain. 2011;134(12):3470–9.

DOI: 10.1080/21678421.2017.1374605/0010

IMG-11 Brain-behaviour correlates of bulbar ALS: gray and white matter regional analyses

S Shellikeri1,2, M Myers1,2, SE Black1,2,3, L Zinman1,2,3, Y Yunusova1,2

1University of Toronto, Toronto, ON, Canada, 2Sunnybrook Research Institute, Toronto, ON, Canada, 3Sunnybrook Health Sciences Center, Toronto, ON, Canada

Email address for correspondence: sanjana.shellikeri@mail.utoronto.ca

Keywords: MRI, DTI, bulbar ALS

Background: Thirty percent of patients with ALS present with bulbar-onset ALS, yet the majority of patients exhibit bulbar disease as ALS progresses. Bulbar ALS is associated with a low quality-of-life, reduced survival and the fastest rate of decline. Bulbar disease may also be associated with cognitive-linguistic deficits. Measures for early identification of bulbar disease remain limited, however. MRI metrics may serve as sensitive biomarkers of early changes in the disease, but are rarely examined in bulbar ALS. This may be because methods for identifying relevant bulbar regions are not well established and the regions within the speech-language network (SLN) that may contribute to bulbar and cognitive-linguistic deficits are unknown. Further, how changes in the SLN areas correlate with speech production dysfunction is not well established. The study objectives are to: 1) develop and clinically validate a novel method of primary motor cortex (PMC) partitioning that allows examination of the bulbar motor region; 2) identify neuroanatomic changes in the SLN areas in ALS; and 3) examine neuroanatomic correlates of bulbar dysfunction.

Methods: T1-weighted (130 slices, res = 1 mm3) and diffusion tensor imaging (DTI) images (36 directions, res = 1.4 mm3) were obtained from 16 patients (7M, M = 61.38 YoA) with varying degrees of bulbar ALS (ALSFRS-R bulbar: M = 10.81, SD = 1.55) and 13 healthy controls (HC; 7M, M = 62.92 years of age). Regions of interest included bilateral bulbar and limb PMC regions and areas in the SLN, such as pars triangularis (parsT), pars opercularis, posterior superior temporal gyrus (pSTG) and transverse temporal gyrus (TTG). Measures included cortical thickness, grey matter (GM) volume, surface area and DTI indices (i.e. fractional anisotropy; FA, mean diffusivity; MD, radial diffusivity; RD, axial diffusivity; AD). Bulbar areas in the PMC were identified using a novel semi-automatic method (Freesurfer). Inter-rater reliability and construct validity were established. Brain-behaviour associations between neuroanatomical indices and bulbar and limb function and disease severity measures were examined using linear mixed-effects (LME) models.

Results: The PMC partitioning method had excellent inter-rater reliability (ICC >0.994) and correlated with clinical measures of motor dysfunction (R2 = 0.710–0.759, p = 0.05–0.008). Group effects indicated WM pathology in left parsT (FA, p = 0.050) and pSTG (AD, p = 0.036). Bulbar motor dysfunction, characterized by articulatory rate, correlated with GM changes in bilateral parsT and
TTG (surface area and GM volume, $p=0.045$–$0.001$). Neither the overall disease severity nor limb motor dysfunction measured by ALSFRS-R and grip strength index (kg/BMI) correlated with extramotor neuroanatomy.

Discussion: Patients with bulbar ALS present with GM and WM changes in the left SLN cortical areas. Further, bilateral neuroanatomic changes in these regions were related to the degree of bulbar motor dysfunction and not with overall disease severity or spinal symptomatology, suggesting bulbar ALS may be pathologically unique with increased extramotor burden and validating the potential use of MRI metrics as markers of bulbar ALS.

DOI: 10.1080/21678421.2017.1374605/0011

IMG-12 Frontostriatal grey matter atrophy in ALS – detection on individual patient level

R Radakovic$^{1,2,3}$, E Flanagan$^4$, M Kiernan$^5$, E Mioshi$^1$, M Hornberger$^3$

$^1$School of Health Sciences, University of East Anglia, Norwich, United Kingdom, $^2$Alzheimer Scotland Dementia Research Centre, $^3$Centre for Cognitive Ageing and Epidemiology; University of Edinburgh, Edinburgh, United Kingdom, $^4$Norwich Medical School, University of East Anglia, Norwich, United Kingdom, $^5$Brain and Mind Centre, University of Sydney, Sydney, Australia

Email address for correspondence: radakovic.ratho@gmail.com

Keywords: visual atrophy rating scale, MRI, medial orbitofrontal cortex

Background: Amyotrophic lateral sclerosis (ALS) is characterised by cortical and sub-cortical changes, with both prefrontal cortex and striatal atrophy being reported (1). Specifically, recent research has shown that ALS patients display significantly higher visually rated atrophy than controls in the orbitofrontal cortex (2). However, all previous work in this direction was conducted on a group level and it is not clear whether such atrophy can also be detected on an individual level via a simple visual atrophy scale in the clinic.

Objective: To explore whether visual atrophy rating on an individual level can detect frontostriatal changes in ALS.

Methods: 21 ALS patients without dementia and 17 controls were recruited from the Sydney ALS clinic and underwent an MRI scan. T1 weighted scans were rated on a 5-point Likert scale, with 0 indicating normal and four indicating severe atrophy (2,3). Examination of focal bilateral regions of interest in patients and controls was undertaken, specifically of the prefrontal cortex, with the orbitofrontal cortex sub-divided to the medial orbitofrontal cortex and lateral orbitofrontal cortex, the anterior cingulate cortex and the striatum, sub-divided to the caudate nucleus and the nucleus accumbens. Within region visual atrophy rating differences were examined using Kruskal-Wallis tests corrected for False Discovery Rate.

Results: There was no significant age difference between ALS patients and controls. On overall prefrontal cortex or the striatum visual atrophy ratings, there was no significant difference between ALS patients and controls. Further sub-division of the prefrontal cortex showed a significantly higher atrophy rating in the bilateral medial orbitofrontal cortex in ALS patients versus controls ($p<0.05$). No difference was observed in the lateral orbitofrontal cortex and the anterior cingulate cortex. Classification of ALS patients based on medial orbitofrontal cortex atrophy showed that patients with higher medial orbitofrontal cortex atrophy tended to have significantly higher caudate nucleus atrophy ($p<0.05$) than those patients with less medial orbitofrontal cortex atrophy.

Discussion and conclusion: Our study has shown that ALS patients show specific and increased atrophy bilaterally in the medial orbitofrontal cortex. Not surprisingly, medial orbitofrontal cortex atrophy was related to striatal atrophy due to the strong ventrostriatal structural and functional connectivity of this region. Overall, we show that, even using a simple visual atrophy scale on an individual level, one can reliably detect frontostriatal deficits specific to ALS. Further research should apply this visual rating method to explore connectivity between cortical and sub-cortical regions, accounting for white matter changes, to allow for comprehensive visual atrophy rating.

References

1. Grolez G, Moreau C, Danel-Brunaud V, et al. BMC Neurology. 2016;16:1–17.
2. Ambikairajah A, Devenney E, Flanagan E, et al. Amyotroph Lateral Scler. 2014;5:226–34.
3. Davies RR, Scabill VL, Graham A, et al. Neuroradiology. 2009;51:491–503.

DOI: 10.1080/21678421.2017.1374605/0012

IMG-13 Voxel-based morphometry (VBM) subcortical white matter changes correlate with D50 model disease progression in amyotrophic lateral sclerosis

M Batyrbekova$^1$, T Prell$^1$, B Stubendorff$^3$, M Bokemeyer$^2$, T Mayer$^2$, V Hartung$^3$, OW Witte$^1$, J Grosskreutz$^1$

$^1$Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany,
$^2$Department of Neuroradiology, Jena University Hospital, Jena, Germany, $^3$Department of Radiology, HELIOS Clinic, Gotha, Germany
**Background:** The cause of heterogeneity in the ALS clinical course is still unclear. Hence, there is an urgent need to identify ALS-related changes in the brain in different ALS sub-types to facilitate an early diagnosis and stratification for clinical trials. Yet, in numerous studies, correlation of anatomical changes in the brain and functional decay in the ALSFRS-R has been poor.

**Objectives:** The aim of this study is to correlate site of onset, disease phases, degree of disability and disease progression rate as ascertained from a new D50 model of ALS disease progression with disease-related changes is gray (GM) and white matter (WM) in a large cohort of well characterized ALS patients (n = 153).

**Methods:** T1-weighted MRI of 90 ALS patients and 63 healthy controls were analyzed. Patients were classified into sub-groups and analyzed to determine changes in GM and WM. To describe the disease course of our cohort we applied our new model including D50 (time when ALSFRS-R drops to 24) and disease phases I-IV. ALSFRS-R, AALSFRS-R and D50 were correlated to WM and GM changes. Disease phases were used to reveal differences in volume-changing patterns within the individual disease course.

**Results:** Using Computational Anatomy Toolbox for SPM (CAT12), rapid progression, late phase and high degree of disability were characterized by pronounced reduction in GM and WM in the central and frontal brain regions, whereas subcortical WM was more severely altered. Furthermore, WM changes correlate significantly with ALSFRS-R, AALSFRS-R and D50. The phases show different patterns of WM changes. In phase I, D50 correlates with WM changes that are localized in subcortical motor regions, corticospinal tract, brainstem and cerebellum. Phase II patients are characterized by a more widespread pattern, indicating a leap of pathology beyond the motor system when transition from early semi-stable disease to a progressing phase occurs.

**Conclusion:** Using Voxel-based Morphometry (VBM) in high resolution T1 in a large cohort of ALS patients, we were able to identify the key regions of ALS pathology which correlated well with three clinical progression parameters. It is likely that previous studies were underpowered and ALS-related heterogeneity induced noise in the clinical parameters, which obscured the relevant pathology in computerized voxel-based analyses.

**Acknowledgments:** This research is supported by BMBF (Bundesministerium für Bildung und Forschung) in the framework of the E-RARE programme (PYRAMID), JPND (OnWebDUALS) of the European Union, and the Dt. Gesellschaft für Muskelkrankung (DGM).

**Keywords:** VBM, progression, D50

---

**IMG-14 Progression of cerebellar involvement in amyotrophic lateral sclerosis as seen by SUIT/CAT12 voxel-based morphometry and D50 disease modelling**

M Batyrbekova1, T Prell1, B Stubendorff4, M Bokemeyer2, T Mayer2, V Hartung3, OW Witte1, J Grosskreutz2

1Hans-Berger-Department of Neurology, Jena University Hospital, Jena, Germany, 2Department of Neuroradiology, Jena University Hospital, Jena, Germany, 3Department of Radiology, HELIOS Clinic, Gotha, Germany

**Email address for correspondence:** beatrice.stubendorff@med.uni-jena.de

**Keywords:** VBM, progression, D50

**Background:** The cerebellum is essential for intact motor function and has an important role in cognitive and neuropsychiatric processes. It is known that the cerebellum is involved in ALS pathology, but the role of gray matter (GM) and white matter (WM) changes is still unclear.

**Objective:** The current study sets out to describe the involvement of the cerebellum with regard to the site of onset, disease phases, degree of disability and disease progression rate in a large cohort of well characterized ALS patients.

**Methods:** T1-weighted MRI of 84 ALS patients and 63 healthy controls were analyzed. Based on these clinical parameters, patients were classified into sub-groups and analyzed to determine changes in GM and WM. D50 is a new parameter to describe the ALS disease course (D50 := time point when ALSFRS-R drops to 24). Based on D50, ALS disease course can be divided in different disease phases (I–IV), each phase covering half of D50. ALSFRS-R, AALSFRS-R and D50 were correlated to MRI data of all ALS patients and patients in phase I and II to show the differences in volume-changing patterns.

**Results:** The mean age of the patients was 60 ± 11.49 years, male:female ratio was 49:35, ALSFRS-R at MRI was 37 ± 7.29, mean AALSFRS-R was 0.68 ± 0.54, average disease duration was 23 ± 30.08 months, D50 was 38.63 ± 32.87 months. The patient group included 24 bulbar and 60 limb onset. 46 patients had a low degree of disability (ALSFRS-R ≥ 38), 38 were highly impaired (ALSFRS-R < 38). In phase I there were 36 patients and in phase II there were 42. Based on AALSFRS-R, we subgrouped patients as rapid (AALSFRS-R > 1.5; n = 9) and slow (AALSFRS-R < 0.5; n = 36). Additionally, the rapid (D50 < 15 months; n = 9) and slow (D50 > 40 months; n = 25) sub-groups were categorized based on D50. Late phase and high degree of disability were characterized by pronounced GM reduction in cerebellar regions, whereas WM alterations were localized primarily in the brainstem. Rapid progressors show more GM atrophy.

**Email address for correspondence:** beatrice.stubendorff@med.uni-jena.de

**Keywords:** VBM, progression, D50

---

**Acknowledgments:** This research is supported by BMBF (Bundesministerium für Bildung and Forschung) (DGM).

**DOI:** 10.1080/21678421.2017.1374605/0013
Conclusion: Using a spatially unbiased atlas template of the cerebellum and brainstem (SUIT) toolbox and a Computational Anatomy Toolbox for SPM (CAT12) in a large cohort of ALS patients, we were able to indicate that the sub-types demonstrate different patterns of GMatrophy in the cerebellum. These results might be important to understand the pathophysiology of ALS. D50 seems to be more sensitive than ΔALSFRS-R and could be used as a progression biomarker in combination with Voxel-based Morphometry (VBM).

Acknowledgments: This research is supported by BMBF (Bundesministerium für Bildung und Forschung) in the framework of the E-RARE programme (PYRAMID), JPND (OnWebDUALS) of the European Union and the Dt. Gesellschaft für Muskelkranke (DGM).

DOI: 10.1080/21678421.2017.1374605/0014

IMG-15 Sensitivity and specificity of neurite orientation dispersion and density magnetic resonance imaging (NODDI) at the single patient level in amyotrophic lateral sclerosis

AW Barritt, R Broad, PN Leigh, M Cercignani

Brighton and Sussex Medical School, Brighton, United Kingdom

Email address for correspondence: awb@doctors.org.uk

Keywords: neuroimaging, NODDI, diagnosis

Background: Neurite Orientation Dispersion and Density Imaging (NODDI) models MRI diffusion to derive neurite density index (NDI) and other parameters. NODDI in patients with amyotrophic lateral sclerosis (ALS) has previously revealed significantly reduced NDI throughout the intracranial corticospinal tracts (CSTs) at group level relative to healthy controls. The aim of this study was to assess the usefulness of NODDI at single subject level, by means of the sensitivity and specificity of a patient-control classifier based on NDI values.

Methods: 24 patients with ALS and 23 healthy controls underwent NODDI scans. Voxel-wise Z-score maps for whole brain NDI were created for all subjects against the group mean of the control images. Masks for the CSTs and corpus callosum (CC) were applied, separately (CST or CC) and in combination (CST + CC), and the number of voxels within these regions were stratified at thresholds of 2, 3 and 4 negative standard deviations (SDs) in each patient and control subject. Receiver operator characteristic (ROC) analysis was performed in MatLab by varying the classification threshold MNV = minimum number of voxels with Z-score <2, 3 or 4.

Results: Voxels with NDI below 3 SDs in all three regions yielded the best overall discrimination between patients and controls, with areas under the ROC curve (AUCs) > 0.9 in CST and CST + CC. Representative sensitivity and specificity values were calculated for selected MNVs. Sensitivity and specificity were, respectively, 83.3% and 95.7% for the CST with MNV = 200; 79.2% and 87% for the CC with MNV = 21; and 96% and 87% for CST + CC with MNV = 80.

Conclusion: The NDI parameter derived from NODDI of the CSTs and CC can distinguish patients with ALS from control subjects with competitive rates of sensitivity and specificity. These findings require further validation and development, but may help establish NODDI as a clinically-useful diagnostic tool for atypical presentations of ALS.

DOI: 10.1080/21678421.2017.1374605/0015

IMG-16 Selective alteration of thalamic motor structural connectivity in ALS

S Tu1,2,3, R Menke2,3, K Talbot3, M Kiernan1, M Turner2,3

1Brain and Mind Centre, Sydney Medical School, University of Sydney, Sydney, Australia, 2Wellcome Centre for Integrative Neuroimaging, 3Nuffield Department of Clinical Neurosciences; University of Oxford, Oxford, United Kingdom

Email address for correspondence: sicong.tu@ndcn.ox.ac.uk

Keywords: MRI, thalamus, biomarker

Background: The thalamus is a major relay structure of the brain involved in modulating and integrating sensory, motor and more widespread cortical processes. The thalamus has been implicated in the pathology of various neurodegenerative conditions, including ALS, which is recognized to be a multi-system cerebral disorder.

Objectives: We examined whether patients with ALS show selective dysfunction in thalamic connectivity to motor cortices (BA4/BA6) and whether there is regional disparity within motor-related thalamo-cortical connectivity.

Methods: Diffusion-weighted and T1-weighted MRI data were available in 20 non-demented ALS patients and 27 healthy age and education matched controls. Masks of frontal, temporal, visual and motor (BA4/BA6) cortices were generated for each individual using FreeSurfer. The thalami of all participants were segmented using FIRST (part of FSL) and parcellated into sub-regions based on local patterns of connectivity with the various cortical masks resulting from probabilistic tractography. Thalamo-cortical tracts were reconstructed using group averaged parcellations of thalamic sub-regions with a threshold of 90% as seed masks. Volumes (corrected for head size) and diffusion tensor imaging
metrics within thalamic sub-regions and corresponding tracts were compared between patients and controls.

**Results:** Bilateral thalamic connectivity to motor cortices was selectively altered in ALS patients compared to healthy controls. Mean diffusivity (MD) and radial diffusivity (RD) of both motor-related thalamic sub-regions and white matter tracts were consistently higher in ALS patients compared to controls ($p<0.035$ and $p<0.005$, respectively). In addition, there was a significant positive correlation between MD and RD of the parcellated BA4 (primary) motor region of the thalamus and disease duration (all $p<0.05$) in ALS patients. There was no significant disparity between the degree of thalamic connectivity alteration to BA4 and BA6 (pre-motor) motor cortices. Thalamic connections to frontal, temporal and visual cortices remained intact in ALS patients.

**Conclusions:** Structural connectivity between the thalamus and motor cortices is selectively altered in ALS, with MD and RD showing the highest sensitivity. Thalamic connectivity deserves further exploration as a source of potentially more holistic neuroimaging biomarkers for ALS cortical pathology.

**Acknowledgments:** This study was supported by the Australian National Health and Medical Research Council (ST), and the Medical Research Council/Motor Neurone Disease Association UK Lady Edith Wolfson Senior Fellowship (MRT).

DOI: 10.1080/21678421.2017.1374605/0016

**Keywords:** spinal cord, DTI, inhomogeneous magnetization transfer

**Background:** Spinal cord (SC) imaging is an emerging area of ALS biomarker research. In this preliminary study, our objective was to investigate the potentiality of high-resolution anatomical imaging, Diffusion Tensor Imaging (DTI) and conventional/inhomogeneous Magnetization Transfer imaging (MT/ihMT) analyzed with template-extracted regions of interest to measure the atrophy and the structural changes of white and gray matter spinal cord (SC) occurring in ALS patients.

**Methods:** Ten patients with a spinal form of ALS and 20 age-matched healthy controls were recruited and scanned at 3T MRI. The SC gray matter (GM) and white matter (WM) areas were automatically segmented using dedicated templates. The atrophy indices were evaluated from T2-weighted images at each vertebral level from cervical C1 to C6. DTI and ihMT metrics were quantified within the corticospinal tracts, the posterior sensory tracts and the anterior GM horns at C2 and C5 levels. The clinical disabilities of patients were evaluated using ALSFRS-R, an Upper Motor Neuron scale and the MRC scale and correlated to the MR metrics.

**Results:** A significant GM atrophy (C4 to C6) and WM atrophy (C1 to C6) were observed in ALS patients compared to healthy controls. The SC atrophy was correlated with decreased ALSFRS-R scores. Interestingly, significant decreases of fractional anisotropy (FA), MT and ihMT ratios, along with an increased radial diffusivity, were found in the regions of interest, namely the lateral corticospinal tract and the anterior GM horns at both C2 and C5 cervical levels, whereas apparent diffusion coefficients (ADC) significantly increased in anterior horns. Strong correlations between MRI metrics and clinical scores were also found.

**Conclusion:** Altogether, these preliminary results suggest that high-resolution anatomical imaging and ihMT imaging, in addition to DTI, are valuable to characterize SC tissue impairment in ALS. In this study, in addition to an important SC WM demyelination, we also observed impairments of cervical anterior GM for the first time in ALS. Further longitudinal studies in a larger population and with refined MR sequences are to be conducted to investigate the prognostic role of GM atrophy and WM demyelination as markers of disease progression and to provide more insights to the pattern of degeneration and underlying processes involved in ALS pathophysiology.

DOI: 10.1080/21678421.2017.1374605/0017
IMG-18 Spinal cord MRI: is it an effective classification tool for the diagnosis of motor neuron disease conditions?

G Querin1, MM El Mendili2, S Delphine3, T Lenglet3, V Marchand-Pauvert1, P-F Pradat5

1Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d’Imagerie Biomédicale, Paris, France, 2Department of Neurology, Icahn School of Medicine, Mount Sinai, USA, 3Institut des Neurosciences Translationnelles, Institut Du Cerveau Et De La Moelle Épinière - IHU-A-ICM, Paris, France, 4APHP, Hôpital Pitie-Salpêtrière, Département de Neurophysiologie, Paris, France, 5Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire d’Imagerie Biomédicale, APHP, Hôpital Pitié-Salpêtrière, Département des Maladies du Système Nerveux, Centre référent SLA, Paris, France

Email address for correspondence: giorgia.querin@gmail.com

Keywords: spinal cord MRI, classification, lower motor neuron diseases

Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving both upper and lower motor neurons. Diagnosis remains largely based on clinical evaluation and EMG and is frequently long delayed from symptom onset. Diagnostic delay may compromise inclusion of the patients in clinical trials and probably reduces efficacy of neuroprotective drugs. Moreover, symptoms at onset of the disease are often overlapping with those referable to ALS-mimic syndromes, such as SMA. Diagnosis more challenging, even for expert clinicians. A classic ALS-mimic syndrome is SMA, which is a genetically determined, autosomal recessive, lower motor neuron disease, usually presenting as a childhood pathology, but whose symptoms may also appear in young adulthood. Recent studies outline possible diagnostic applications based on brain MRI pattern recognition using machine learning and data mining, which seem promising and could help in making the diagnostic process more effective.

Objective: The aim of this study was to test a classification approach based on cervical spinal cord MRI metrics for differentiation of ALS patients from controls and patients affected by a well-defined ALS-mimic syndrome such as SMA.

Methods: We studied 56 ALS patients, 17 adult SMA patients (type 3–4) and 47 healthy controls (HC) matched for sex and age. Each subject underwent cervical spinal cord MRI using a 3T MRI system. Spinal cord cross-sectional area at each vertebral level was computed. Diffusion tensor imaging (DTT) metrics were measured (fractional anisotropy; FA, mean diffusivity; MD, radial diffusivity; RD, axial diffusivity; AD). Magnetization transfer ratio (MTR) was extracted. Patients were subdivided into three groups for age normalization (18–40, 40–60 and 60–80 years). A random forest classifier algorithm with bootstrap technique was applied. Receiver operator characteristic (ROC) curve and Area under the curve (AUC) were computed to test diagnostic abilities.

Results: This algorithm gave a diagnostic ability between ALS and HC with AUC of 0.99 and between ALS and SMA of 0.99. FA was the best classification feature both between ALS and HCs and between ALS and SMA patients.

Conclusion: FA resulted to be a reliable classification feature between ALS and SMA patients and between patients and controls. Such a result is interesting under a pathological point of view, underlying differences in the pathogenesis of diverse motor neuron diseases. Moreover, this study opens the road to the use of automated diagnostic applications based on spinal cord imaging in the study of ALS patients. Wider populations are needed for robust validation of the tool.

DOI: 10.1080/21678421.2017.1374605/0018

IMG-19 Ultra-short echo time magnetic resonance spectroscopy of multiple metabolites in amyotrophic lateral sclerosis, preliminary findings

J Blicher1, T Staermose1, K Figlewski1, AT Møller2, J Near3

1Center of Functionally Integrative Neuroscience, Aarhus University, Aarhus, Denmark, 2Department of Neurology, Aarhus University Hospital, Aarhus, Denmark, 3Douglas Mental Health University Institute and Department of Psychiatry, McGill University, Montreal, Canada

Email address for correspondence: jbl@cfin.au.dk

Keywords: magnetic resonance spectroscopy, GABA, glutamate

Background: Brain metabolites such as N-acetylaspartate (NAA), GABA, Glutamate, Glutathione and Myo-Inositol have been suggested as part of the pathophysiology of ALS. We aimed to investigate the usefulness of ultra-short echo Magnetic Resonance Spectroscopy (MRS) as a diagnostic tool and biomarker of disease progression in ALS. Spin Echo Full Intensity Acquired Localized (SPECIAL) spectroscopy allows for simultaneous quantification of all of the above-mentioned metabolites (1). The purpose of the current study was to investigate the feasibility and spectral quality of SPECIAL MRS in ALS patients.

Methods: Patients with ALS (possible, probable or definite) were recruited and scanned once on a 3T
Magnetom Tim Trio system, using SPECIAL spectroscopy (TE = 8.5 ms, TR = 4000 ms). A 20×20×20 mm voxel was placed in the hand area of the primary motor cortex, corresponding to the side of initial symptom onset with a MRS scan time of around 11 minutes. Metabolites of interest were quantitated as a ratio of Creatine (Cr) using LCModel analysis software.

**Results:** The study is still ongoing. Here we present our preliminary results from five male patients (aged 54–74, all treated with riluzole) and compare to nine healthy subjects (five male, mean age 65) scanned in a prior study using the same MRS sequence and voxel placement. The obtained spectra were of a sufficient quality (SNR 31–55) to allow for quantification of metabolites of interest. Compared to healthy subjects, several metabolites were significantly altered in patients. Specifically, we found an increase in Myo-Inositol/Cr (p = 0.02) and a decrease in NAA/Cr (p = 0.01) and Glutamate/Cr (p = 0.003) in ALS patients compared to healthy subjects. We found no difference in GABA/Cr (p = 0.30) or Glutathione/Cr (p = 0.09) values between the two groups.

**Discussion:** The finding of elevated Myo-Inositol and low NAA is in agreement with most prior studies. The finding of low Glutamate level and normal GABA levels is surprising, as prior studies have found Glutamate/Glutamine to be elevated and GABA to be low, in line with the hypothesis of excitotoxicity in ALS (2,3). Our finding of low Glutamate/Cr could be due to atrophy and loss of excitatory neuron in the voxel of interest. Further analysis is needed to correct for grey matter content within the voxel.

**Conclusion:** SPECIAL MRS is a promising new method that allows for quantification of metabolites related to both excitatory/inhibitory balance, oxidative stress and neuronal integrity and, thus, can provide insights into several of the possible underlying pathologies of ALS. Further studies are needed to clarify the usability of the method in early diagnostics and to compare to results from other diseases mimicking ALS.

**References**
1. Near J, et al. NMR Biomed. 2013;26:1353–1362.
2. Han J, et al. J Magn Reson Imaging. 2010;31:305–308.
3. Foerster, et al. Neurology. 2012;20:1596–1600.

DOI: 10.1080/21678421.2017.1374605/0019

**IMG-20 Elemental imaging of post-mortem CNS in MND using laser ablation-ICP-MS**

K Kysenius1,2, B Paul3, D Hare2,4, P Crouch1

1Department of Pathology, University of Melbourne, Melbourne, Australia, 2Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, 3School of Earth Sciences, University of Melbourne, Melbourne, Australia, 4Elemental Bio-imaging Facility, University of Technology, Sydney, Australia

**Email address for correspondence:** kai.kysenius@unimelb.edu.au

**Keywords:** laser ablation ICP-MS, elemental imaging, copper

**Background:** The elemental composition of all biological material reflects the complexity arising from millions of molecules participating in millions of chemical reactions. Specific differences in the relative abundance of these elements and their biochemical and anatomical partitioning gives each tissue a unique ‘elemental signature’. Measuring perturbations in the natural elemental signature of a given tissue can provide valuable insight to the cause of disease and potential opportunity for therapeutic intervention (1). Within the narrow confines of just one component of the elemental signature, our recent work outlines the role of copper malfunction in MND (2) and has detailed the protective effects of the copper-containing drug CuII(atm) in mouse models of MND (3) (now in clinical trials NCT02870634). Broader understanding of the elemental signature of MND, across multiple regions of the central nervous system (CNS), is likely to expedite targeted therapy development.

**Objectives:** To identify changes in the natural elemental signature of different CNS regions affected by MND.

**Methods:** We used laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) to assess anatomically defined changes to magnesium, manganese, iron, copper, zinc, calcium, sulphur and rubidium in fresh frozen tissue sections (spinal cord, thalamus, motor cortex, cerebellum and occipital cortex) from MND cases (n = 6–30) and controls (n = 6–20). This technique involves vapourising material with a laser, which is transferred into an ICP-MS via argon gas. The quantitative analysis software allows 2D reconstruction of anatomical elemental maps.

**Results:** Our analyses revealed significant elemental changes in the spinal cord, motor cortex and occipital cortex between control and MND cases. In the spinal cord, the ventral horns presented with decreased copper levels (p = 0.048) and increased iron levels (p = 0.0434) compared to controls. Additionally, zinc levels were increased throughout the spinal cord (p = 0.013). In the motor cortex, both iron (p = 0.0012) and zinc (p = 0.0322) were increased in the grey matter, whereas only iron (p = 0.0451) was increased in the white matter. In the occipital cortex, rubidium levels were decreased in the white matter (p = 0.0494).

**Discussion and conclusion:** Our analysis of human MND CNS tissue revealed significant anatomical elemental changes, primarily in the spinal cord and motor cortex, two primary sites of MND pathology. We hypothesise that these localised changes to copper, zinc and iron distribution reflect biochemical alterations of the metalloproteins and offer multiple avenues for therapeutic intervention.

**Acknowledgments:** We acknowledge the Sigrid Juselius Foundation, NHMRC, ABC, Motor Neurone Disease Research Institute of Australia (Betty Laidlaw MND Grant), and the Victorian Brain Bank Network for funding and support of our research.
IMG-21 Relationship between brain metabolism and cognitive/behavioral functioning in ALS

J De Vocht¹, SMA Willekens², J Evens¹, D Van Weehaeghe², K Van Laere², P Van Damme¹,³

¹Division of Neurology, ²Division of Nuclear Medicine and Molecular Imaging; University Hospitals Leuven and KU Leuven, Leuven, Belgium, ³KU Leuven, University of Leuven, Department of Neurosciences, Center for Brain and Disease Research, Laboratory of Neurobiology, Leuven, Belgium

Email address for correspondence: joke.devocht@uzleuven.be

Keywords: cognition, imaging, ECAS

Background: Previous research classified ALS as a multisystem disorder in which problems outside the motor system are also present. Over the last decade, a strong clinical and molecular link between ALS and the neurodegenerative disorder frontotemporal dementia (FTD) has been uncovered as about 10–15% of ALS patients display co-morbid FTD (ALS-FTD), while up to 50% display mild cognitive or behavioral impairment. Previous studies have suggested that ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) Positron Emission Tomography (PET) could be an early diagnostic marker of cognitive impairment in ALS. ¹²³I-GE180 compared to [¹⁸F]-PBR28 in detecting in vivo glial activation in people with ALS

MJ Alshikho¹,², NR Zürcher¹, P Cernasov², B Reynolds², S Babu², L Marinelli³, R Carter³, D Yokell⁴, G Elfakhrid⁴, D Wooten⁴, M Normandin⁴, J Masdeu⁵,⁶

¹A. A. Martinos Center for Biomedical Imaging, Department of Radiology, ²Neurological Clinical Research Institute (NCRI), Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, USA, ³GE Global Research, Niskayuna, USA, ⁴Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital, Boston, USA, ⁵Houston Methodist Neurological Institute, Houston, USA, ⁶Department of Neurology, Weill Cornell Medicine, New York, USA

Email address for correspondence: malshikho@mgh.harvard.edu
Background: $[^{18}F]$-GE180 and $[^{11}C]$-PBR28 are different radiotracers that both bind to translocator protein (TSPO), which is expressed on reactive glial cells. People with ALS show increased $[^{11}C]$-PBR28 uptake in the motor cortices that is highly correlated with clinical phenotype (1,2). $[^{18}F]$-GE180 has a longer half-life than $[^{11}C]$-PBR28, which could allow for radiolabelling and distribution from regional radiopharmacies to facilitate multi-center studies. This is the first study to use $[^{18}F]$-GE180 PET imaging in people with ALS.

Objective: To compare radiotracer uptake in the motor cortices in people with ALS imaged with both $[^{18}F]$-GE180 and $[^{11}C]$-PBR28 positron emission tomography (PET).

Methods: Eight ALS participants and eight healthy controls (HC) underwent $[^{18}F]$-GE180 PET scans. Six of those participants (5 ALS and 1 HC) were also scanned using $[^{11}C]$-PBR28 PET within 1 month of the $[^{18}F]$-GE180 PET scan. PET images were acquired 60–90 minutes after radiotracer injection and the standardized uptake value ratio (SUVR) normalized to whole brain was used to quantify the tracer’s uptake in the brain. A composite region of interest (ROI) has been formed from the precentral gyri bilaterally and its continuation on the medial surface of the hemisphere (ie the paracentral gyrus). SUVR-GE180 and SUVR-$[^{11}C]$-PBR28 were compared between ALS and HC within the ROI.

Results: The signal captured by $[^{11}C]$-PBR28 PET in the motor cortices of people with ALS was higher and more informative than $[^{18}F]$-GE180 PET. In the ROI, the mean (SD) of SUVR-$[^{11}C]$-PBR28 in 5 ALS participants was 1.05 (0.042), while the mean (SD) of SUVR-GE180 in the same 5 ALS participants was significantly lower (0.96 (0.031); p<0.05). A comparison in SUVR-GE180 between 8 ALS individuals and 8 HC within the ROI revealed no difference between the groups (HC: 0.95 ± 0.09; ALS: 0.96 ± 0.03; p = 0.67).

Conclusion: $[^{18}F]$-GE180 PET is not as sensitive as $[^{11}C]$-PBR28 PET in imaging glial activation in people with ALS.

References
1. Zürcher NR, et al. NeuroImage: Clinical. 2015;7:409–14.
2. Alshikho MJ, et al. Neurology. 2016;87(24):2554–61.

DOI: 10.1080/21678421.2017.1374605/0022

IMG-23 PET imaging studies show enhanced expression of mGluR5 and inflammatory response during progressive degeneration in ALS mouse model expressing SOD1-G93A gene

A-L Brownell¹, D Kuruppu¹, K-E Kil¹, K Jokivarsi¹, P Poutiainen¹,², A Zhu¹, M Maxwell¹

¹Massachusetts General Hospital, Boston, USA,
²Kuopio University Hospital, Kuopio, Finland

Email address for correspondence: pekka.poutiainen@uef.fi

Keywords: PET

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative motor neuron disorder. Genetic studies have linked mutation of the gene SOD1 to ALS pathology as well as several other pathological processes including modulation of glutamatergic function and inflammatory processes. Since therapeutic approaches for ALS are focused on glutamatergic function, we investigated modulation of glutamate transport based on its receptor function as well as excitotoxicity-induced inflammatory response.

Methods: In vivo positron emission tomography (PET) imaging studies of metabotropic glutamate receptor subtype 5 (mGluR5) using $[^{18}F]$FPEB and inflammatory response using $[^{11}C]$PBR28 were done in an early and a late phase of neurodegeneration in four ALS mice expressing SOD1-G93A gene and four control base mice (C57/BL6). Accumulation of $[^{18}F]$FPEB and $[^{11}C]$PBR28 were quantitated in several brain areas and spinal cord to determine degeneration-induced modulation. The studies were completed with immunohistochemical analyses of mGluR5 and inflammatory response.

Results: These studies showed enhanced binding potential of $[^{18}F]$FPEB in several brain areas including the striatum, hippocampus and frontal cortex. In the whole brain, the binding potential increased 49 ± 9% from base mice to ALS-type mice and further enhanced 23 ± 4% during disease progression. Also, in the spinal cord, 6–22% enhanced accumulation of $[^{18}F]$FPEB was observed during progression of the disease. The accumulation of $[^{11}C]$PBR28 increased by 110 ± 33% in the whole brain during progression of the disease, indicating a significant inflammatory process. $[^{11}C]$PBR28 accumulation enhanced 89–264% in the spinal cord and 204% in the lungs. The end-point immunohistochemical analyses verified the enhanced mGluR5 expression and inflammation.

Conclusion: These results confirm the role of glutamate and inflammation in ALS-type pathology. These data also support the hypothesis that excessive glutamate may contribute to inflammation in the chronic neurodegenerative processes in ALS.

DOI: 10.1080/21678421.2017.1374605/0023
**IMG-24 Development of positron emission tomography radiotracer for imaging cannabinoid receptor type 2 (CB2) in ALS**

M Weber¹, R Slavik², AM Herde², A Haider², S Krämer², R Schibli², S Ametamey², L Mu²

¹Neuromuscular Diseases Unit/ALS Clinic, St.Gallen, Switzerland, ²Institute of Pharmaceutical Sciences, ETH Zürich, Zürich, Switzerland

Email address for correspondence: markus.weber@kssg.ch

Keywords: spinal cord, PET, cannabinoid type II receptor (CB2)

**Background:** The endocannabinoid system is a highly preserved system with so far two well-characterized G protein-coupled receptors type I (CB1) and type II (CB2). Under physiological conditions CB2 receptor expression in the brain and spinal cord is barely detectable. In contrast, in ALS mouse models CB2 receptor expression in spinal cord is increasingly upregulated with disease progression and stimulation of the CB receptor with CB2 agonists exerts profound neuroprotective effects, even when administered at symptom onset. It is not known whether up-regulation of CB2 receptors also plays a role in human ALS.

**Objective:** To develop a positron emission tomography radioligand for CB2 imaging in human ALS and to demonstrate CB2 expression human spinal cord sections.

**Methods:** Three novel fluorinated 4-oxo-quinoline derivatives were designed, synthesized and pharmacologically evaluated. The most potent candidate (N-(1-adamantyl)-1-(2-(2-fluoroethoxy)ethyl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxamide (denoted [¹⁸F] RS-126) was obtained in ≥99% radiochemical purity with an average specific radioactivity of 98 GBq/µmol at the end of the radiosynthesis. In vitro autoradiographic studies were performed with CB2-positive rat spleen tissue and human spinal cord sections as control and human ALS spinal cord sections from three different levels (cervical, thoracic and lumbar).

**Results:** Rat spleen tissue and ALS spinal cord sections at all three levels revealed high binding of [¹⁸F]RS-126. Uptake of [¹⁸F]RS-126 to CB2-positive spleen tissue of rats was blockable, suggesting specific binding to CB2 receptors. For human ALS spinal cord tissues, a higher accumulation of [¹⁸F]RS-126 was observed compared to spinal cord sections from healthy controls.

**Conclusion:** [¹⁸F]RS-126 is a promising PET ligand for imaging CB2 receptor expression in human ALS. This study provides evidence that CB2 receptor expression is up-regulated at all levels of the spinal cord in ALS patients.

DOI: 10.1080/21678421.2017.1374605/0024

**IMG-25 Diaphragm ultrasound in ALS: a case demonstrating an important role for this technique**

M Ross, I Muzyka, J Dalrymple, B Miller, M Burge, A Chang, B Smith, B Estephan

Mayo Clinic Arizona, Scottsdale, USA

Email address for correspondence: ross.mark@mayo.edu

Keywords: phrenic nerve conduction studies, diaphragm ultrasound, diaphragm pacing

**Background:** Diaphragm pacing is an experimental ALS treatment, available through a compassionate use program. Eligibility for diaphragm pacing requires forced vital capacity (FVC) between 45–50% predicted and phrenic nerve conduction study (NCS) evidence showing the diaphragm can be electrically stimulated. Diaphragm ultrasound also evaluates diaphragm function by demonstrating thickening with inspiration.

**Objectives:** To demonstrate diaphragm ultrasound provides information about diaphragm function that can serve to supplement or even countermand phrenic NCS results.

**Case study:** A 63 year old man with upper motor neuron predominant ALS requested diaphragm pacing treatment as his respiratory functions worsened. He was wheelchair-bound and had severe dysarthria and dysphagia. He had exertional dyspnea and used continuous positive airway pressure at night for obstructive apnea.

**Results:** FVC was 47% predicted. Initial phrenic NCS showed a normal response on the right, but no response on the left. The absent left phrenic NCS response made him ineligible for diaphragm pacing. Diaphragm function was further assessed with diaphragm ultrasound. This showed normal thickening with inspiration bilaterally. The diaphragm ultrasound result prompted repeating the left phrenic NCS, which then showed a normal response. He was then eligible to undergo the diaphragm pacing procedure. He successfully completed surgical implantation of diaphragm leads for diaphragm pacing. At surgery, both diaphragms showed good responses to electrical stimulation.

**Conclusion:** Phrenic NCS can be technically challenging to perform. In some patients, it may be difficult to locate the optimal stimulation site for the phrenic nerve in the neck. Likewise, in some patients, it may be difficult to place surface recording electrodes close to the diaphragm muscle. For these reasons, it may be possible that phrenic NCS show no response, even though the phrenic nerve may be working well. In this patient, diaphragm ultrasound indicated good diaphragm function, which prompted repeating phrenic NCS. The normal phrenic NCS on repeat testing allowed the patient to pursue diaphragm pacing. Diaphragm ultrasound could be considered an alternative technique for demonstration of preserved diaphragm function.

DOI: 10.1080/21678421.2017.1374605/0025