



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis

Citation for published version:

Muller, H-P, R Turner, M, Grosskreutz, J, Abrahams, S, Bede, P, Govind, V, Prudlo, J, Ludolph, AC, Filippi, M & Kassubek, J 2016, 'A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis' *Journal of Neurology, Neurosurgery & Psychiatry*, vol. 87, no. 6, pp. 570-579. DOI: 10.1136/jnnp-2015-311952

Digital Object Identifier (DOI):

[10.1136/jnnp-2015-311952](https://doi.org/10.1136/jnnp-2015-311952)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Journal of Neurology, Neurosurgery & Psychiatry

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



**A LARGE-SCALE MULTI-CENTRE CEREBRAL DIFFUSION TENSOR IMAGING STUDY
IN AMYOTROPHIC LATERAL SCLEROSIS**

¹Hans-Peter Müller, ²Martin R. Turner, ³Julian Grosskreutz, ⁴Sharon Abrahams, ⁵Peter Bede, ⁶Varan Govind, ⁷Johannes Prudlo, ¹Albert C Ludolph, ⁸Massimo Filippi, ¹Jan Kassubek
for The Neuroimaging Society in ALS (NiSALS) DTI Study Group

¹Department of Neurology, University of Ulm, Ulm, Germany

²University of Oxford Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, United Kingdom

³Hans-Berger Department of Neurology, Jena University Hospital, Jena, Germany

⁴Human Cognitive Neuroscience, Psychology-PPLS & Euan MacDonald Centre for MND Research & Centre for Cognitive Aging and Epidemiology, University of Edinburgh, Edinburgh, United Kingdom

⁵Quantitative Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland.

⁶Department of Radiology, University of Miami School of Medicine, Miami, Florida, United States of America

⁷Department of Neurology, University of Rostock and DZNE, Rostock, Germany

⁸Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy

Corresponding author: Prof. Dr. Jan Kassubek
Department of Neurology, University of Ulm
Oberer Eselsberg 45, 89081 Ulm, Germany
phone + 49 731 1771206
email jan.kassubek@uni-ulm.de

Running title: Multi-centre DTI in ALS

Key words: Biomarker, diffusion tensor imaging, motor neuron disease, neurodegenerative disease, neuroimaging

Word count: 2931

ABSTRACT

Objective: Damage to the cerebral tissue structural connectivity associated with amyotrophic lateral sclerosis (ALS), which extends beyond the motor pathways, can be visualized by diffusion tensor imaging (DTI). The effective translation of DTI metrics as biomarker requires its application across multiple magnetic resonance imaging scanners and patient cohorts. A multi-centre study was undertaken to assess structural connectivity in ALS at a large sample size.

Methods: Four-hundred-and-forty-two DTI data sets from patients with ALS (N=253) and controls (N=189) were collected for this retrospective study from eight international ALS-specialist ~~international~~ clinic sites. Equipment and DTI protocols varied across the centres. Fractional anisotropy (FA) maps of the control subjects were used to establish correction matrices to pool data, and correction algorithms were applied to the FA maps of the control and ALS patient groups.

Results: Analysis of data pooled from all centres using whole-brain-based statistical analysis of FA maps confirmed the most significant alterations in the corticospinal tracts, and captured additional significant white matter tract changes in the frontal lobe, brainstem and hippocampal regions of the ALS group that coincided with *post mortem* neuropathological stages. Stratification of the ALS group for disease severity (ALS functional rating scale) confirmed these findings.

Interpretation: This large-scale study overcomes the challenges associated with processing and analysis of multi-platform, multi-centre DTI data, and effectively demonstrates the anatomical fingerprint patterns of changes in a DTI metric that reflect distinct ALS disease stages. This success paves the way for the use of DTI-based metrics as read-out in natural history, prognostic stratification and multi-site disease-modifying studies in ALS.

INTRODUCTION

The use of advanced magnetic resonance imaging (MRI) techniques, in particular diffusion tensor imaging (DTI) of white matter tracts, has greatly improved the understanding of the *in vivo* cerebral and spinal neuropathology of the adult neurodegenerative disorder amyotrophic lateral sclerosis (ALS).[1-5] DTI can quantify the integrity of large white matter tracts *in vivo* using metrics such as fractional anisotropy (FA).[6] A DTI-based *in vivo* imaging concept has also been applied to the recently introduced neuropathological staging system, indicating that ALS may disseminate in regional patterns.[7,8] ALS overlaps with frontotemporal dementia both clinically and pathologically. While the majority of ALS patients do not develop a frank dementia, a large proportion show cognitive impairments on the same spectrum, and DTI has demonstrated extension of white matter changes into frontal and temporal lobes accordingly.[9,10]

Imaging biomarkers are urgently required for future pharmaceutical trials to be used as objective study end-points. The development of robust prognostic and diagnostic markers has become a major research priority in this notoriously heterogeneous disorder.[11,12] Effective biomarkers, particularly those integrated in therapeutic studies, need to be applicable across multiple international centres that, in case of MRI, may vary in their scanner hardware and sequence acquisition parameters.

The Neuroimaging Society in ALS (www.nisals.org) was established in 2010 and developed a roadmap for the standardisation and harmonisation of advanced MRI data in ALS.[13] The most sensitive cerebral pathology found in cross-sectional MRI studies in ALS patients has been in the white matter tracts,[14] in contrast to the characteristic hippocampal atrophy that largely defines Alzheimer's Disease. To date, there have been few large-scale multi-site DTI studies, and none in ALS. Data analysis of such studies may be hampered by differences in scanning protocols,[15,16] thus an approach to pool DTI data acquired with different protocols was sought. The objective was to identify ALS induced alterations in the white matter structural connectivity in a large scale patient cohort from multiple sites spread across the world.

To this end, a new strategy has been developed that paves the way to use the large numbers of DTI data sets from local data bases of different study sites for comprehensive large-scale multicentre imaging studies.

METHODS

Subject populations and scanning protocols

Four-hundred-and-forty-two DTI data sets from patients with ALS (N=253) and control subjects (N=189) from eight ALS-specialist clinic sites (tertiary referral centres) were selected *ex post facto* [for this retrospective study](#) (Dublin, Ireland; Edinburgh, UK; Jena, Germany; Miami, USA; Milano, Italy; Oxford, UK; Rostock, Germany; Ulm, Germany). Centre specific details, including number of subjects and DTI scanning protocols, are given in **Table 1**. [Patients' data were randomly selected for inclusion as they were available in the respective data bases of each site.](#) All patients were diagnosed with ALS [according to the revised El Escorial criteria \[17,18\]](#) according to standard clinical criteria by experienced ALS neurologists. [Upper motor neuron signs were present in all patients.](#) No patient had frank dementia. Severity of disease-related physical symptoms was measured using the revised ALS functional rating scale (ALS-FRS-R) [179], and it ranged from 14 to 48 with a mean of 37 ± 7 (96% ascertainment).

Table 1. Number and demographics of subjects and DTI scanning protocols of the multi-centre setting. Age and voxelsize influence directly FA. Indirect influence on FA maps (via signal-to-noise ratio of recorded DTI data sets) results from the number of gradient directions (GD), the field strength (B0) and echo time (TE). *Data using two different protocols from the same centre were treated separately.

centre	<u>ALS</u> (m/f)	<u>mean age /</u> <u>years</u>	<u>controls</u> (m/f)	<u>mean age</u> <u>/ years</u>	<u>TR /</u> <u>s</u>	<u>TE /</u> <u>ms</u>	<u>vsize (x/y/z)</u> <u>/ mm</u>	<u>vsize /</u> <u>mm³</u>	<u>no.</u> <u>GD</u>	<u>b /</u> <u>s/mm²</u>	<u>B0 / T</u>
<u>01</u>	<u>27 (18/9)</u>	<u>61</u>	<u>17</u> (8/9)	<u>58</u>	<u>7.8</u>	<u>97</u>	<u>1.3/1.3/2.5</u>	<u>3.9</u>	<u>33</u>	<u>1000</u>	<u>1.5</u>
<u>02</u>	<u>50</u> (29/21)	<u>60</u>	<u>26</u> (11/15)	<u>49</u>	<u>10.0</u>	<u>94</u>	<u>2.0/2.0/2.0</u>	<u>8.0</u>	<u>31</u>	<u>1000</u>	<u>3.0</u>
<u>03a*</u>	<u>28 (17/11)</u>	<u>57</u>	<u>14</u> (8/6)	<u>57</u>	<u>8.0</u>	<u>93</u>	<u>1.5/1.5/2.2</u>	<u>5.0</u>	<u>13</u>	<u>800</u>	<u>1.5</u>
<u>03b*</u>	<u>22 (8/14)</u>	<u>64</u>	<u>17</u> (9/8)	<u>62</u>	<u>8.0</u>	<u>95</u>	<u>2.0/2.0/2.8</u>	<u>11.2</u>	<u>52</u>	<u>1000</u>	<u>1.5</u>
<u>04</u>	<u>18 (10/8)</u>	<u>65</u>	<u>20</u> (9/11)	<u>64</u>	<u>5.1</u>	<u>85</u>	<u>2.0/2.0/2.0</u>	<u>8.2</u>	<u>92</u>	<u>1000</u>	<u>3.0</u>
<u>05</u>	<u>48</u> (27/21)	<u>61</u>	<u>49</u> (27/22)	<u>59</u>	<u>7.6</u>	<u>59</u>	<u>2.2/2.2/2.5</u>	<u>12.0</u>	<u>34</u>	<u>1100</u>	<u>3.0</u>
<u>06</u>	<u>21 (14/7)</u>	<u>54</u>	<u>20</u> (9/10)	<u>51</u>	<u>11.8</u>	<u>80</u>	<u>1.1/1.1/2.2</u>	<u>2.7</u>	<u>52</u>	<u>1000</u>	<u>3.0</u>
<u>07</u>	<u>18 (6/12)</u>	<u>61</u>	<u>16</u> (9/7)	<u>60</u>	<u>9.0</u>	<u>80</u>	<u>0.9/0.9/2.5</u>	<u>2.2</u>	<u>33</u>	<u>1100</u>	<u>3.0</u>
<u>08</u>	<u>21 (11/10)</u>	<u>59</u>	<u>10</u> (6/4)	<u>66</u>	<u>16.5</u>	<u>98</u>	<u>2.0/2.0/2.0</u>	<u>8.0</u>	<u>71</u>	<u>1000</u>	<u>1.5</u>
<u>total or</u> <u>mean</u>	<u>253</u> (140/113)	<u>60</u>	<u>189</u> (96/93)	<u>58</u>	<u>n.a.</u>	<u>n.a.</u>	<u>n.a.</u>	<u>n.a.</u>	<u>n.a.</u>	<u>n.a.</u>	<u>n.a.</u>

Ethics statements

Following the NiSALS governance guidelines, all data uploaded to the NiSALS repository were fully deidentified. As a safety measure against accidental disclosure of personal information, the data were run through a deidentification routine again after upload to the repository which followed the recommendations of the NEMA organization (<http://medical.nema.org>) in DICOM PS 3.15 2011

– Security and Systems Management

(ftp://medical.nema.org/medical/dicom/2011/09v11dif/09v11_15.doc)

which lists the DICOM header fields which contain personal and protected information, including

centre and scanner identifying information. These items were removed from the headers and new global unique identifiers were generated for each image slice. Data were then pooled and released to the analysis group. Therefore, the data analyzed in this study contained no identifiable or protected information accessible to the investigators.

The local Ethics committees (EC) and specific EC reference numbers of all centres contributing data to this study, which allow for the sharing of fully deidentified data with scientific collaborators such as the exemption 4 of the 45 CFR 46 for the University of Miami site, were as follows;

1) Department of Neurology, University of Ulm, Ulm, Germany: The Ethics Committee of the University of Ulm: reference 19/12

2) Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom: South Central Oxford Ethics Committee: reference 08/H0605/85

3) Hans-Berger Department of Neurology, Jena University Hospital, Jena, Germany: The ethics committee of the Friedrich-Schiller-University Jena: reference 3633-11/12

4) Human Cognitive Neuroscience, Psychology-PPLS & Euan MacDonald Centre for MND Research & Centre for Cognitive Aging and Epidemiology, University of Edinburgh, Edinburgh, United Kingdom: NHS Scotland A Research Ethics Committee: REC reference 08/MRE00/50

5) Quantitative Neuroimaging Group, Academic Unit of Neurology, Trinity College Dublin, Dublin, Ireland: Ethics (Medical Research) Committee - Beaumont Hospital, Dublin, Ireland: reference 08/90

6) Department of Radiology, University of Miami School of Medicine, Miami, Florida, United States of America: The University of Miami Institutional Review Board (IRB) approved the NIH-funded study: Brain MR Imaging and Spectroscopy of Amyotrophic Lateral Sclerosis, reference eProst ID: 20043623

7) Department of Neurology, University of Rostock and DZNE, Rostock, Germany: Medical Ethics Committee of Rostock University Medical Centre: reference A 2011 56

8) Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience,

San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy: Ethical Committee of the Ospedale San Raffaele, Milano: reference RF-2010-2313220.

Preprocessing and whole-brain-based voxelwise comparison

Data from each centre were assessed for completeness and, according to an established analysis quality control,[18] corrupted gradient directions in single DTI data sets were excluded from further analysis.[2019] The correction of eddy current-induced geometric distortions of the echo-planar imaging-based DTI data sets was performed prior to further analysis.[21] Control for motion artefacts were~~was~~ also performed by use of the quality control algorithm.[20] That way, specific image artefacts such as susceptibility-induced geometric warping, physiological and bulk patient motion, and chemical shift artefacts could be removed from the single data sets from further processing. The application of this quality control and repair algorithm allowed that DTI data sets that contained some corrupted gradient directions did not have to be excluded from the study. Out of the 442 data sets in total, 27 had to undergo the quality repair function.

Following a standardized iterative stereotaxic normalization process, using study specific DTI template sets,[1822] FA maps of all participating subjects were derived centrewise in Montreal Neurological Institute (MNI) stereotaxic standard space.

Cross-sectional group comparison analysis followed the procedure described in detail previously.[2218] Maps of FA were calculated from MNI-normalized DTI data, and a Gaussian smoothing filter of 8 mm FWHM was applied to the normalized individual FA maps. The filter size of 8 mm (which is about 2-3 times the recording voxel size, depending on the protocol) provides a good balance between sensitivity and specificity.[23,2419-21] Then, voxelwise statistical comparison (whole-brain-based spatial statistics, WBSS) was performed between the ALS patient group and the corresponding control group (refer to subsection “statistics”).~~by Student's t-test with correction for multiple comparisons using the false discovery rate (FDR) algorithm [22] at $p < 0.05$~~

~~and a clustering procedure at a threshold cluster size of 512 voxels to reduce type I and type II errors.[21] Voxels with FA values below 0.2 were not considered for statistical analyses since cortical gray matter shows FA values up to 0.2.[23] Centerwise cross-sectional results are provided in Supplementary Figure 1.~~ Single-centre cross-sectional comparisons have already been reported in various studies.[24,25]

For the comparison of centres, a study-specific template set (b0 and FA) was created with equal weighting of centres, ALS-patients, and controls; then stereotaxic normalization was performed repeatedly with these equally weighted templates.

Influencing factors of FA maps

Different factors may contribute to the variability of DTI data of control subjects and ALS patients. Although the precise influence of each source of variation could not be delineated, investigating group FA differences between patients and controls on systematic between-centre differences was used to decide whether pooling across centres was feasible.[24] Potential sources of variability directly or indirectly influence DTI metrics. Data from the eight centres differed especially in mean age of the subjects and in recorded voxel size. These two parameters directly influence FA, which is known to be decreased in elderly subjects [26,27] and, especially in complex fibre-tracking structures with different axonal directionalities, FA is decreased with larger voxel size.[28] Further parameters like field strength (B0), echo time (TE), and number of gradient directions (GD) influence the signal-to-noise-ratio and thus indirectly influence FA-values. Furthermore, centre-specific sources of variability on DTI metrics, e.g. scanner specific variability, environmental noise, specific factors such as scanning time might be present in single centre studies but will only slightly influence comparisons at the group level.[29] Therefore, a strategy to regress out confounders in a two-step procedure was applied to controls' data.

First, the covariates age, voxel size, TE, number of GD, and B0 were regressed out in FA maps of controls, and a corrected FA-map set consisting of FA maps of all controls was derived (**Figure 1**).

Corrections for asymmetrical voxel size were not performed as the orientation of the scans in different sites was not identical. In a second step, comparison of FA maps of controls was performed centrewise and, if the number of significant voxels in the group comparison of controls was below a threshold of 10,000 voxels, centres were merged into centre-clusters. Finally, residual centre-specific influences (physiological and susceptibility artefacts, timing of scan, phenotype variations within the diagnosis of ALS, centre-specific environmental noise, etc.) were defined together as inter-centre effects. In a final step, 3-D linear correction matrices were calculated by computing the voxelwise differences of averaged FA values for the different centres.[30] Due to the limited number of control data sets of some centres, a voxelwise linear regression calculation [30] with the respective 3-D regression correction matrices was not possible. The linear 3-D corrections were then ~~and~~ applied to the ~~FA maps of data sets from~~ each centre (**Figure 1**).~~[29]~~

Centrewise pooling of ALS patients' FA maps

For ALS patients' FA maps, the covariates voxel size, age, number of GD, ~~B0~~field strength, and TE were regressed out with the 3D covariate regression matrices that were derived from the controls' FA maps and centre clusters were set up according to the classification derived for controls' FA maps. Afterwards, 3D linear correction matrices for centre clusters were applied (**Figure 1**). A split-half stability test (splitting the eight centres to reach almost a split-half for ALS patients and controls) was performed, comparing the results clusters of centres 03, 04, 06, 07, 08 (128 ALS patients vs. 98 controls) and the results clusters of centres 01, 02, 05 (125 ALS-patients vs. 91 controls).

Statistics

WBSS was performed voxelwise by Student's t-test with correction for multiple comparisons using the false-discovery-rate (FDR) algorithm [31] at $p < 0.05$ and a clustering procedure at a threshold cluster size of 512 voxels to reduce type I and type II errors.[24] Voxels with FA values below 0.2

were not considered for statistical analyses since cortical gray matter shows FA values up to 0.2.[32] Voxelwise correlations between FA values and clinical scores (ALS-FRS-R) were calculated using Pearson correlation; corrections for multiple comparisons were performed using the FDR algorithm at $p < 0.05$ followed by a clustering procedure at a threshold cluster size of 512 voxels. FA-based regions-of-interest (ROI) analysis was performed by arithmetically averaging FA values of individual data sets within a spherical ROI of radius 10 mm. Voxels with FA values below 0.2 were not considered for statistical analyses. Comparisons between FA values of ALS patients and controls were performed by Student's t-test.

RESULTS

Single-centre voxelwise comparison of FA maps (WBSS)

The whole-brain-based voxelwise analysis of single centres' FA maps without correction of confounding multicentric factors showed FA decreases mainly localized along the CST. From these single-centre analyses, no substantial alterations beyond the CST were detected. Centrewise cross-sectional results are provided in **Figure 2**.

Voxelwise correction of confounding factors

FA maps of controls showed significant correlations ($p < 0.05$, corrected for multiple comparisons) to the covariates voxel size, age, number of GD, B0field strength, and TE (**Supplementary Figure 1A, upper panel**). After regressing out these covariates, only small clusters of significant correlation to the aforementioned covariates remained. The application of the correction matrices to FA maps of ALS patients also reduced the dependency on the covariates voxel size, age, number of GD, B0field strength, and TE (**Supplementary Figure 1A, lower panel**). Centres were merged into centre-clusters. That way centres 3b and 6 form a centre-cluster, and centres 3a, 5, and 7 form the reference centre cluster (**Supplementary Figure 1B**). Residual accompanying centre-specific influences in between centre clusters were corrected by 3D linear correction matrices using centre-

cluster C as a reference (**Supplementary Figure 1C**). WBSS of centre clusters are provided in Figure 2B.

The whole-brain-based analysis was performed both for uncorrected and for corrected FA maps of 253 ALS patients and 189 controls and demonstrated difference maps as displayed in **Figure 3**. Resulting alteration patterns were apparently very similar, but after correction a more symmetric pattern between hemispheres was revealed; furthermore, no FA increase clusters remained and the resulting cluster showed an increased interconnectivity. Group comparison of corrected FA maps showed in total a number of approximately 170,000 voxels significant whereas the group comparison of uncorrected FA maps revealed only approximately 140,000 voxels (Figure 3).

Multi-site region-of-interest (ROI) analysis of FA maps

FA map analysis was performed in several ~~regions of interest (ROIs)~~ that were located in white matter structures prone to be affected in the course of ALS; [7] ~~the CST, frontal white matter structures, brainstem, and hippocampal area.~~ Effect size in terms of significance of differences ~~increased according to the pathological spreading pattern predicted by post mortem pathology (Supplementary Figure 2)~~ According to atlas-based MNI coordinates, ROI localizations were determined in the upper and central CST (MNI $\pm 22/-23/42$ and MNI $\pm 23/-22/1$, respectively), in the frontal white matter (MNI $\pm 20/5/42$ and MNI $\pm 23/-26/7$), in the brainstem (MNI $\pm 12/-21/-15$), and in the hippocampal area (MNI $\pm 36/-19/-14$). This analysis was performed in the centrewise corrected and harmonized FA maps of 253 ALS patients and 189 controls. The effect size in terms of significance of differences was the higher the earlier the respective anatomical ROI structure was expected to be involved in the degenerative process according to the pathological spreading pattern predicted by post mortem pathology (Supplementary Figure 2A). In addition, effect sizes (p-values) for the different ROIs ~~were~~ are illustrated in **Supplementary Figure 2B**, separately for the different centres. As for the whole data set, most significant results were observed along the CST for all single centres, followed by clusters in the frontal ROIs and the ROIs in the brainstem and the

hippocampal area.

Multi-site voxelwise comparison of FA maps (WBSS)

The whole-brain-based analysis was performed for corrected FA maps of 253 ALS patients and 189 controls demonstrated difference maps as displayed in **Figure 34**. Differences were connected within one large cluster covering an area of about 1570,000 mm³ in the white matter $p < 0.05$, corrected for multiple comparisons; this number of significant voxels was higher than computed by WBSS of “uncorrected” FA-maps (approx. 140,000 m³). A split-half stability test (splitting the eight centres to reach almost a split-half for ALS patients and controls) was performed, comparing the results clusters of centres 03, 04, 06, 07, 08 (128 ALS patients vs. 98 controls) and the results clusters of centres 01, 02, 05 (125 ALS patients vs. 91 controls), revealing a similar number of difference cluster voxels for both groups (Figure 2C). The split-half test as well as the whole group analysis showed basically the same brain regions to be affected (although with a different number of cluster voxels): ~~W~~with respect to the tract specific analysis,[8] major significances were found along the corticospinal tracts (CST, corresponding to neuropathological stage 1 [7]), including the “horseshoe” configuration (in coronal slicing) reflecting superior CST and transcallosal interconnecting fibres,[303] frontal involvement including areas crossed by the corticopontine and corticorubral tracts (neuropathological stage 2), and the corticostriatal pathway (pathological stage 3), pathways to brainstem (pontine/rubral involvement, pathological stage 2), and hippocampal areas including the proximal portion of the perforant path (pathological stage 4) (**Figure 43A**).

With the application of more lenient thresholding, a pattern resembling the neuropathological spreading could be demonstrated, beginning with highest significances (corrected $p < 0.00005$, N=23500) in the upper CST, followed by clusters along the CST (corrected $p < 0.00045$, N=46000), then including frontal areas (corrected $p < 0.0045$, N=83500), and, at lowest significances level at (corrected $p < 0.005$ (N=162200) also including brain stem as well as hippocampal areas (**Figure**

43B). This pattern could also be replicated by uncorrected data with a reduced number of significant results voxels, i.e. at corrected $p < 0.00005$: $N=15300$, at corrected $p < 0.0005$: $N=34300$, at corrected $p < 0.005$: $N=61600$, and at corrected $p < 0.05$: $N=134600$ (**Supplementary Figure 3**). Following the theory of spreading patterns that could be expressed by ALS-stages [7,8], relations were investigated between the FA values and a clinical disability score, i.e. the ALS-FRS-R. For this task, ~~stratification~~the samples of ALS patients were stratifiedas undertaken according to clinical disability at the time of scanning. Three age- and gender-matched groups which were homogeneously distributed over centres were selected, obtaining identical group size and gender ratio and no significant differences in age distribution: a-38 patients with ALS-FRS-R ranging from 44 to 48 (m/f 20/18, mean age 6258 years), 38 patients with ALS-FRS-R ranging from 31 to 43 (m/f 20/18, mean age 5862 years), and 38 patients with ALS-FRS-R ranging from 14 to 30 (m/f 20/18, mean age 59 years) (**Figure 5**). The group with mild ALS-FRS-R decrease showed a summed-up cluster of 11366400 mm³ to be affected as significant FA decrease, the group with moderate ALS-FRS-R decrease showed in sum 26575600 mm³ to be affected, and the group with moderate ALS-FRS-R decrease showed in sum 617070 mm³ of regional FA decreases.

After complete-corrections for covariates and centre differences, a significant voxelwise correlation of FA values and ALS-FRS-R ~~were~~as observed along the CST ($p < 0.05$ $R > 0.4$, corrected for multiple comparisons, **Supplementary-Figure 45B**). No correlations were found for frontal areas as well as for brainstem and hippocampal regions. NB: correlation analysis for uncorrected FA maps did not show any significant clusters for correlations ~~between~~ FA and ALS-FRS-R (**Supplementary Figure 1**).

DISCUSSION

This pooled analysis of multi-site MRI data demonstrated extensive motor and extramotor white matter tract pathology in a large number of ALS patients compared to healthy controls. This is an important step in the development of DTI-derived biomarkers of neuropathology which are

applicable across multiple sites. FA is a marker of cerebral tract damage, demonstrated in multiple studies across a range of disorders, and so a natural candidate biomarker for ALS. It is a long way from formal validation as such, but if it is to be feasible as an outcome measure in a multi-centre trial then it will be necessary to assess across a large number of individuals from different centres and scanners. This is the first time such an analysis has been undertaken.

Previous imaging studies have investigated ALS-related alterations of white matter with group sizes of typically 15-30 patients and healthy controls.[2,5] These studies have consistently detected reduced FA (and concurrently increased radial diffusivity) within the rostral corticospinal tracts and commissural callosal fibres. The current study using an unprecedented number of subjects has revealed more widespread changes. By altering the significance threshold for group differences, a disease-specific pathological pattern of regional involvement was identified which is consistent with *post mortem* histopathology findings.[34] A deeper exploration of the data in relation to phenotype would be a logical next step but requires the provision of more detailed clinical information. The ALSFRS-R score is based upon physical (motor) activities of daily living and is not obviously sensitive to frontal or hippocampal (non-motor) involvement. Nonetheless, mild ALS-FRS-R compared to moderate and severe ALS-FRS-R demonstrated pathological extension from pure CST involvement to involve the wider white matter.

Factors known to directly influence the FA are the voxel size [289] and participant age.[267,278] Indirect influencing factors on FA (via the signal-to-noise-ratio) are the field strength, the number of gradients, the pulse sequence, and further centre specific factors. In DTI, multi-site studies appear to be the best solution in order to improve the statistical power in investigation. Recent DTI studies reported replicability, reliability, and stability of DTI-based FA measurements in multi-site environments with common acquisition protocols. These studies include a study with 26 patients with Alzheimer's Disease and 12 controls on 16 scanners,[16] a study with 9 controls on 2 scanners,[15] and a study with 2 controls on 5 scanners.[352] Additional studies with harmonized DTI protocols have already been performed in Alzheimer's disease as well as in Huntington's

disease.[336,347] A framework for the analysis of phantom data in multicentre DTI studies has been provided previously.[385] Reproducibility of DTI metrics has been recently tested in a sample of healthy controls at two identical scanners [396] reporting that the within and between session reproducibility was lower than the values for intersubject variability. The initial suggestions on how to correct for differences in FA maps with different protocols were recently published.[3029,4037] Common acquisition protocols but involving different subjects were investigated for groupwise FA differences between patients and controls on systematic between-centre differences.[374] During the pooling process, a number of well-defined parameters that confound the FA results could be regressed out. Nevertheless, to some extent, residual centre-specific FA-influencing factors might be identified. That way, matrices for regressing out FA-influencing factors could be applied in the identical manner to FA-maps of ALS-patients. The comparison between the analysis of corrected and the analysis of uncorrected data showed a difference of about 20% in the number of significant voxels. However, as in this study the ratio patients/controls was approximately equal between the centres, the correction effects were less prominent as it could be expected if the ratio patients/controls had differed more between the centres. Furthermore, this study shows that affectations could be observed at the multicentre level with high subject numbers that would not have been possible to show in single centre studies with low subject numbers. ~~Furthermore, this study shows that at the multicentre level with high subject numbers affectations appeared that would not have been possible to show in single centre studies with low subject numbers.~~

This methodological framework could easily be adapted to further DTI metrics, e.g. radial, axial, or mean diffusivity.

In summary, this study has demonstrated that it is possible to meaningfully interpret combined DTI data from different MRI manufacturers and software platforms after application of appropriate compensations for centre-specific differences. This might pave the way to repurpose larger numbers of DTI data sets of ALS patients for more clinically comprehensive large-scale multicentric imaging studies.

A limitation of this study compared to other multicentre studies of neurodegenerative diseases (e.g. the PADDINGTON study in Huntington's Disease or the Alzheimer's Disease Neuroimaging Initiative (ADNI)[[4138,4239](#)]) is the heterogeneous nature of DTI data resource in terms of magnetic field strength and acquisition sequence parameters used across the centres. It would be advantageous in a future prospective multi-site study to try to harmonise as many data acquisition sequence parameters as possible. A further potential limitation of the current approach is the limited number of control data sets per centre which may be not homogeneous over centres, and might lead to false or over-correction. However, N=20 (which is the case in seven out of the eight centres) has been a standard control sample size in single-centre studies.[[330,403,414](#)] As the ratio patients/controls did not differ strongly between different centres, a systematic centre difference would be averaged out in the multi-site group comparison.

By this multi-centre approach involving DTI datasets from more than 250 patients, it was possible to reveal the *in vivo* pathoanatomy of ALS non-invasively; the alteration pattern was found to be in agreement with *post mortem* neuroanatomical studies [[3842,425](#)] so that DTI seems to be prone to expand the potential of other neuroimaging markers like fluorodeoxyglucose-positron-emission tomography in ALS.[[463,447](#)] The post mortem cerebral white matter changes in ALS have been long recognised [45,48] but direct correlation with DTI is still in development.[49] This study represents a framework for mapping ALS-specific white matter tract alterations using DTI, and provides encouragement for its extension to other neurodegenerative diseases. In other diseases with emerging evidence of a consistent pathological pattern of spread,[[4550](#)] the white matter tract pathology will be closer to the underlying pathology than clusters of regional atrophy, and thus the DTI metrics have the potential to serve as read-outs and biomarkers of disease and its progression for future clinical trials.

ACKNOWLEDGEMENTS

NiSALS contributors:

Abdulla, Susanne, Department of Neurology, Hannover Medical School, Germany

Agosta, Federica, Neuroimaging Research Unit, Scientific Institute San Raffaele, Milan, Italy

Ajroud-Driss, Senda, Neurology, Northwestern University, USA

Atassi, Nazem, Neurology, Massachusetts General Hospital, USA

Bastin, Mark, Brain Imaging Research Centre, Centre for Cognitive Aging and Epidemiology, University of Edinburgh, Edinburgh, United Kingdom

Benatar, Michael, Dept. of Neurology, University of Miami School of Medicine, USA

Brooks, William, Hoglund Brain Imaging Center, University of Kansas Medical Center, USA

Calvo, Andrea, Rita Levi Montalcini Department of Neuroscience, University of Torino, Italy

Cardenas-Blanco, Arturo, Plasticity and Neurodegeneration, DZNE, Germany

Chio, Adriano, University of Turin, Torino, Italy

De Carvalho, Mamede, Instituto de Medicina Molecular, Lisbon, Portugal

Dahnke, Robert, Department of Psychiatry, University Hospital Jena, Jena, Germany

Enzinger, Christian, Department of Neurology, Medical University of Graz, Graz, Austria

Ferraro, Pilar Maria, Neuroimaging Research Unit, Scientific Institute San Raffaele, Milan, Italy

Floeter, Mary Kay, SSPU, EMG section, National Institute of Neurological Disorders and Stroke, US

Foerster, Bradley, Radiology, Division of Neuroradiology, University of Michigan, USA

Gaser, Christian, Department of Psychiatry, Friedrich-Schiller-University of Jena, Jena, Germany

Geraldo, Ana Filipa, Neuroradiology, HSM, Portugal

Gorges, Martin, Department for Neurology, University of Ulm, Ulm, Germany

Grehl, Torsten, Kliniken Bergmannsheil, Bochum, Germany

Groen, Georg, Section Neuropsychology and Functional Imaging, Department of Psychiatry, University of Ulm, Germany

Hardiman, Orla, Department of Neurology, Trinity College Dublin, Dublin, Ireland

Hartung, Viktor, Department of Neurology, University Hospital Jena, Jena, Germany

Jelsone-Swain, Laura, Department of Psychology, University of South Carolina Aiken, USA

Jenkins, Tom, Department of Neurology, Sheffield Institute for Translational Neuroscience, United Kingdom

Kalra, Sanjay, Medicine (Neurology), University of Alberta, Canada

Kasper, Elisabeth, , University of Rostock, Rostock, Germany

Kitzler, Hagen, Dept. of Neuroradiology, Technische Universitaet Dresden, University Hospital, Dresden, Germany

Koritnik, Blaz, Institute of Clinical Neurophysiology, University Medical Center Ljubljana, Slovenia

Kuzma-Kozakiewicz, Magdalena, Medical University of Warsaw, Poland

LaFleur, Karl, Neurology/Psychiatry, University Medical Center - Utrecht, The Netherlands

Lulé, Dorothée, Neurology, University of Ulm, Ulm, Germany

Machts, Judith, Neurology, German Center for Neurodegenerative Diseases, DZNE, Rostock, Germany

Meoded, Avner, EMG, NINDS, USA

Pettit, Lewis, Human Cognitive Neuroscience, Psychology-PPLS & Euan MacDonald Centre for MND Research & Centre for Cognitive Aging and Epidemiology, University of Edinburgh, Edinburgh, United Kingdom

Pioro, Erik, Neurology, Cleveland Clinic, USA

Poletti, Barbara, Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Italy

Pradat, Pierre-Francois, Department of Neurology, Pitie-Salpetriere Hospital, France

Prell, Tino, Department of Neurology, University Hospital Jena, Jena, Germany

Proudfoot, Malcolm, Clinical Neuroscience, University of Oxford, United Kingdom

Ratti, Elena, Neurology - Neurological Clinical Research Institute (NCRI), Massachusetts General Hospital, USA

Riva, Nilo, Neurology, San Raffaele Scientific Institute, Italy

Robberecht, Wim, Vlaams Instituut voor Biotechnologie, Leuven, Belgium

Ropele, Stefan, Department of Neurology, Medical University of Graz, Graz, Austria

Salachas, Francois, Assistance Publique-Hopitaux de Paris, France

Schmidt, Ruben, Neurology, UMC Utrecht, The Netherlands

Schmidt-Wilcke, Kliniken Bergmannsheil, Bochum, Germany

Schuster, Christina, Trinity College Dublin, Ireland

Shaw, Pamela, Sheffield Institute for Translational Neuroscience, Sheffield, United Kingdom

Sherman, Alex, Neurological Clinical Research Institute, Massachusetts General Hospital, USA

Silani, Vincenzo, Department of Neurology-Stroke Unit and Department of Neurology and Laboratory of Neuroscience, IRCCS Istituto Auxologico Italiano, Dino Ferrari Center, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

Spinelli, Edoardo Gioele, Neurimaging Research Unit, Institute of Experimental Neurology, San Raffaele Scientific Institute, Italy

Teipel, Stefan, Psychosomatik, University of Rostock and German Center for Neurodegenerative Diseases (DZNE), Rostock, Germany

Van Damme, Philip, Department of Neurology, KU Leuven, Belgium

Van den Berg, Leonard, University Medical Center Utrecht, The Netherlands

Van den Heuvel, Martin, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands

Verstraete, Esther, Neurology, University Medical Center Utrecht, The Netherlands

Walhout, Renée, Neurology, University Medical Center Utrecht, The Netherlands

Welsh, Robert, Radiology and Psychiatry, University of Michigan, USA

Weber, Markus, Kantonsspital St.Gallen, Switzerland,

Westeneng, Henk-Jan, Neurology, University Medical Center Utrecht, The Netherlands

Wittstock, Matthias, Department of Neurology, University of Rostock and DZNE, German Center for Neurodegenerative Disorders, Rostock, Germany

Yunusova, Yana, Speech-Language Pathology, University of Toronto, Canada

Funding

The data acquisition at the University of Miami was funded by the National Institutes of Health (USA) grant R01 NS060874.

The data acquisition at the University of Ulm was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG Grant Number LU 336/15-1) and the German Network for Motor Neuron Diseases (BMBF 01GM1103A).

The image acquisition in Edinburgh was performed at the Brain Research Imaging Centre, University of Edinburgh, a center in the SINAPSE Collaboration and was funded by the Silvia Aitken Charitable Trust.

The image acquisition in Milan is granted by the Italian Ministry of Health (Grant #RF-2010-2313220).

The project was supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (Project grant SOPHIA): France, Agence Nationale de la Recherche (ANR); Germany, Bundesministerium für Bildung und Forschung (BMBF); Ireland, Health Research Board (HRB); Italy, Ministero della Salute; The Netherlands, The Netherlands Organisation for Health Research and Development (ZonMw); Poland, Narodowe Centrum Badań i Rozwoju; Portugal, Fundação a Ciência e a Tecnologia; Switzerland, Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung (SNF); United Kingdom, Medical Research Council (MRC).

Conflicts of interest:

None of the authors reported a biomedical interest or potential conflicts of interest. All authors report no disclosures.

REFERENCES

- [1] Agosta F, Chiò A, Cosottini M, et al. The present and the future of neuroimaging in amyotrophic lateral sclerosis. *AJNR Am J Neuroradiol* 2010;31:1769-77.
- [2] Bede P and Hardiman O. Lessons of ALS imaging: Pitfalls and future directions - A critical review. *Neuroimage Clin* 2014;4:436-43.
- [3] Kassubek J, Ludolph AC, Müller HP. Neuroimaging of motor neuron diseases. *Ther Adv Neurol Disord* 2012;5:119-27.
- [4] Keil C, Prell T, Peschel T, et al. Longitudinal diffusion tensor imaging in amyotrophic lateral sclerosis. *BMC Neurosci* 2012;13:141-52.
- [5] Turner MR, Agosta F, Bede P, et al. Neuroimaging in amyotrophic lateral sclerosis. *Biomark Med* 2012;6:319-37.
- [6] Le Bihan D, Mangin JF, Poupon C, et al. Diffusion tensor imaging: concepts and applications. *J Magn Reson Imaging* 2001;13:534-46.
- [7] Brettschneider J, Del Tredici K, Toledo JB, et al. Stages of pTDP-43 pathology in amyotrophic lateral sclerosis. *Ann Neurol* 2013;74:20-38.
- [8] Kassubek J, Müller HP, Del Tredici K, et al. Diffusion tensor imaging analysis of sequential spreading of disease in amyotrophic lateral sclerosis confirms patterns of TDP-43 pathology. *Brain* 2014;137:1733-40.
- [9] Pettit LD, Bastin ME, Smith C, et al. Executive deficits, not processing speed relates to abnormalities in distinct prefrontal tracts in amyotrophic lateral sclerosis. *Brain* 2013;136:3290-304.
- [10] Sarro L, Agosta F, Canu E, et al. Cognitive functions and white matter tract damage in amyotrophic lateral sclerosis: a diffusion tensor tractography study. *AJNR Am J Neuroradiol* 2011;32:1866-72.
- [11] Turner MR, Kiernan MC, Leigh PN, et al. Biomarkers in amyotrophic lateral sclerosis. *Lancet Neurol* 2009;8:94-109.

Formatted: Danish

Formatted: English (United Kingdom)

Formatted: Finnish

Formatted: English (United States)

Formatted: English (United Kingdom)

[12] Turner MR, Benatar M. Ensuring continued progress in biomarkers for amyotrophic lateral sclerosis. *Muscle Nerve* 2015;51:14-8.

[13] Turner MR, Grosskreutz J, Kassubek J, et al. Towards a neuroimaging biomarker for amyotrophic lateral sclerosis. *Lancet Neurol* 2011;10:400-3.

Formatted: English (United Kingdom)

[14] Menke RA, Körner S, Filippini N, et al. Widespread grey matter pathology dominates the longitudinal cerebral MRI and clinical landscape of amyotrophic lateral sclerosis. *Brain* 2014;137:2546-55.

[15] Vollmar C, O'Muircheartaigh J, Barker GJ, et al. Identical, but not the same: intra-site and inter-site reproducibility of fractional anisotropy measures on two 3.0T scanners. *Neuroimage* 2010;51:1384-94.

Formatted: Danish

[16] Teipel SJ, Reuter S, Stieltjes B, et al. Multicentre stability of diffusion tensor imaging measures: a European clinical and physical phantom study. *Psychiatry Res* 2011;194:363-71.

[17] Brooks BR, Miller RG, Swash M, et al. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000;1:293-9.

Formatted: Danish

[18] Ludolph A, Drory V, Hardiman O, et al. A revision of the El Escorial criteria - 2015. *Amyotroph Lateral Scler Frontotemporal Degener* 2015;16:291-2.

Formatted: English (United Kingdom)

[19] Cedarbaum JM, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci* 1999;169:13-21.

[20] Müller HP, Süßmuth SD, Landwehrmeyer GB, et al. Stability effects on results of diffusion tensor imaging analysis by reduction of the number of gradient directions due to motion artifacts: an application to presymptomatic Huntington's disease. *PLoS Curr*. 2011 PMID:22307262.

[21] Shen Y, Larkman DJ, Counsell S, et al. Correction of high-order eddy current

[induced geometric distortion in diffusion-weighted echo-planar images. *Magn Reson Med* 2004;52:1184-9.](#)

[21][22] Müller HP, Kassubek J. Diffusion tensor magnetic resonance imaging in the analysis of neurodegenerative diseases. *J Vis Exp* 2013;77.

[22][23] Müller HP, Unrath A, Huppertz HJ, et al. Neuroanatomical patterns of cerebral white matter involvement in different motor neuron diseases as studied by diffusion tensor imaging analysis. *Amyotroph Lateral Scler* 2012;13:254-64.

[23][24] Müller HP, Kassubek J, Grön G, et al. Impact of the control for corrupted diffusion tensor imaging data in comparisons at the group level: an application in Huntington disease. *Biomed Eng Online* 2014;13:128.

[24][25] Foerster BR, Welsh RC, Feldman EL. 25 years of neuroimaging in amyotrophic lateral sclerosis. *Nat Rev Neurol* 2013;9:513-24.

[25][26] Foerster BR, Dwamena BA, Petrou M, et al. Diagnostic accuracy of diffusion tensor imaging in amyotrophic lateral sclerosis: a systematic review and individual patient data meta-analysis. *Acad Radiol* 2013;20:1099-106.

[26][27] Salat DH, Tuch DS, Greve DN, et al. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. *Neurobiol Aging* 2005;26:1215-27.

[27][28] Lim S, Han CE, Uhlhaas PJ, Kaiser M. Preferential Detachment During Human Brain Development: Age- and Sex-Specific Structural Connectivity in Diffusion Tensor Imaging (DTI) Data.. *Cereb Cortex*. 2015;25:1477-89.

[28][29] Oouchi H, Yamada K, Sakai K, et al. Diffusion anisotropy measurement of brain white matter is affected by voxel size: underestimation occurs in areas with crossing fibers. *AJNR Am J Neuroradiol* 2007;28:1102-6.

[29][30] Roskopf J, Müller HP, Dreyhaupt J, et al. Ex post facto assessment of diffusion tensor imaging metrics from different MRI protocols: preparing for multicentre studies in ALS. *Amyotroph Lateral Scler Frontotemporal Degener* 2015;9:1-10.

Formatted: Danish

[30][31] Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 2002;15:870-8.

[31][32] Künimatsu A, Aoki S, Masutani Y, et al. The optimal trackability threshold of fractional anisotropy for diffusion tensor tractography of the corticospinal tract. *Magn Reson Med Sci* 2004;3:11-7.

Formatted: Finnish

[32][33] Filippini N, Douaud G, Mackay CE, et al. Corpus callosum involvement is a consistent feature of amyotrophic lateral sclerosis. *Neurology* 2010;75:1645-52.

[33][34] Braak H, Brettschneider J, Ludolph AC, et al. Amyotrophic lateral sclerosis - a model of corticofugal axonal spread. *Nat Rev Neurol* 2013;9:708-14.

Formatted: Dutch (Netherlands)

[34][35] Fox RJ, Sakaie K, Lee JC, et al. A validation study of multicenter diffusion tensor imaging: reliability of fractional anisotropy and diffusivity values. *AJNR Am J Neuroradiol* 2012;33:695-700.

Formatted: English (United Kingdom)

[35][36] Jovicich J, Marizzoni M, Bosch B, et al. Multisite longitudinal reliability of tract-based spatial statistics in diffusion tensor imaging of healthy elderly subjects. *Neuroimage* 2014;101:390-403.

[36][37] Müller HP, Grön G, Sprengelmeyer R, et al. Evaluating multicentre DTI data in Huntington's disease on site specific effects: An ex post facto approach. *Neuroimage Clin* 2013;2:161-7.

Formatted: Danish

[37][38] Walker L, Curry M, Nayak A, et al. Brain Development Cooperative Group. A framework for the analysis of phantom data in multicenter diffusion tensor imaging studies. *Hum Brain Mapp* 2013;34:2439-54.

[38][39] Veenith TV, Carter E, Grossac J, et al. Inter subject variability and reproducibility of diffusion tensor imaging within and between different imaging sessions. *PLoS One* 2013;8:e65941.

[39][40] Dyrba M, Ewers M, Wegrzyn M et al. Robust automated detection of microstructural white matter degeneration in Alzheimer's disease using machine learning classification of

multicenter DTI data. *PLoS One* 2013;8:e64925.

[40][41] Hobbs NZ, Cole JH, Farmer RE, et al. Evaluation of multi-modal, multi-site neuroimaging measures in Huntington's disease: Baseline results from the PADDINGTON study. *Neuroimage Clin.* 2012;2:204-11.

[41][42] Nir TM, Jahanshad N, Villalon-Reina JE, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI). Effectiveness of regional DTI measures in distinguishing Alzheimer's disease, MCI, and normal aging. *Neuroimage Clin* 2013;3:180-95.

[42][43] Agosta F, Pagani E, Petrolini M, et al. Assessment of white matter tract damage in patients with amyotrophic lateral sclerosis: a diffusion tensor MR imaging tractography study. *AJNR Am J Neuroradiol* 2010;31:1457-61.

[43][44] Chiò A, Pagani M, Agosta F, et al. Neuroimaging in amyotrophic lateral sclerosis: insights into structural and functional changes. *Lancet Neurol* 2014;13:1228-40

[44][45] Smith MC. Nerve fibre degeneration in the brain in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 1960;23:269-82.

[45][46] Van Laere K, Vanhee A, Verschueren J, et al. Value of 18fluorodeoxyglucose-positron-emission tomography in amyotrophic lateral sclerosis: a prospective study. *JAMA Neurol* 2014;71:553-61.

[46][47] Foerster BR, Feldman EL. A brighter future for patients with amyotrophic lateral sclerosis through imaging? *JAMA Neurol* 2014;71:539-40.

[48] Brownell B, Oppenheimer DR, Hughes JT. The central nervous system in motor neurone disease. *J Neurol Neurosurg Psychiatry* 1970;33:338-57.

[47][49] Miller KL, Stagg CJ, Douaud G, et al. Diffusion imaging of whole, post-mortem human brains on a clinical MRI scanner. *Neuroimage* 2011;57:167-81.

[48][50] Jucker M, Walker LC. Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. *Nature* 2013;501:45-51.

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Formatted: Danish

