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SUMMARY

The aim of this study was to investigate the extent of the cortical and sub-cortical lesions by using in combination voxel-based diffusion tensor imaging (DTI) and voxel-based morphometry (VBM). We included 15 patients with definite or probable ALS and 25 healthy volunteers. Patients were assessed with the revised ALS functional rating scale (ALSFRS-R). In patients, reduced fractional anisotropy was found in bilateral corticospinal tracts, left insula/ventrolateral premotor cortex, right parietal cortex and thalamus, which correlated with the ALSFRS-R. Increased mean diffusivity (MD) was found bilaterally in the motor cortex, the ventrolateral premotor cortex/insula, the hippocampal formations and the right superior temporal gyrus, which did not correlate with the ALSFRS-R. VBM analysis showed no changes in white matter but widespread grey matter volume decrease in several regions exhibiting MD abnormalities. In ALS patients, our results show that subcortical lesions extend beyond the corticospinal tract and are clinically relevant.

INTRODUCTION

In amyotrophic lateral sclerosis (ALS), the extension of the cortical lesions and their correlations with the motor dysfunction remains debated.

Diffusion tensor imaging (DTI) studies using regions of interest (ROIs) method showed a bilateral reduction in anisotropy along the corticospinal tracts (CST).[1-8] Its correlation with motor disability is still debated.[1,3,4,8] Studies using voxel-based method reported essentially a reduced anisotropy in the CST.[9,10]

Voxel-based morphometry (VBM) studies provided conflicting results. Grey matter (GM) reduction found in several regions of the frontal lobe [11-14] was not confirmed by others.[15] Volume reduction [15] or increased density [12] of white matter (WM) were found in motor and non motor regions, but not detected by others.[9,11,13,14]

The present study used a whole brain voxel-based DTI to study mean diffusivity (MD) and fractional anisotropy (FA) in combination with VBM to assess both GM and WM morphological changes. The extent of lesion was correlated with the degree of motor disability assessed by a validated functional scale, the revised ALS functional rating scale (ALSFRS-R).[16]

METHODS

Patients and controls

Fifteen ALS patients (nine men and six women; mean age: 51.8 ± 8.7 years, range: 37 to 69; mean disease duration: 30.9 ± 15.9 months, range: 11 to 66 months) with definite (n=9) or probable (n=6) (http://wfnals.org) sporadic ALS were included. No patients fulfilled the clinical criteria of fronto-temporal lobe dementia. The site of onset was bulbar in four patients, the upper limbs in five patients and the lower limbs in six patients. The mean ALSFRS-R score was 30 ± 6 (range 16 to 36). The control group included 25 healthy volunteers with no history of neurological disorders (11 women and 14 men, mean age: 44.9 ± 12.4 years, range from 31 to 68, difference not significant for age compared to the patients). The study was approved by the local ethics committee and in accordance with the declaration of Helsinki. Informed written consent to participation in the study was obtained from all patients and healthy volunteers.

Imaging protocol

Conventional MRI and DTI data were acquired on a 1.5 T scanner (GE, Milwaukee, USA). Patients underwent structural T_1-weighted (Inversion Recovery – Fast SPGR) and T_2-FLAIR images that were reviewed to exclude potential abnormalities in control subjects. For T_1-weighted images, 124 axial slices were obtained using the following parameters: TR: 10.3 ms, TE: 2.1 ms, inversion time: 400 ms, flip angle: 10^o, acquisition matrix: 256 x 192, reconstruction matrix: 256 x 256, FOV: 24 x 18 cm, in plane resolution 0.937 x 0.937 mm^2, slice thickness: 1.5 mm, no gap.

Diffusion-weighted spin-echo echo planar images (EPI) were acquired with standard head coil for signal reception. Twenty axial slices were obtained using the following parameters: TR: 6500 ms, TE: 85 ms, flip angle: 90^{o}, acquisition matrix: 128 x 128, reconstruction matrix: 256 x 256, FOV: 32 x 32 cm, in plane resolution 1.25 x 1.25 mm^2, slice thickness: 5 mm, no gap. Diffusion weighting was performed along 23 optimized noncolinear directions. A single b value of 700 s/mm^2 was applied. A reference image with no diffusion weighting was also obtained (b_0 image). Raw diffusion-weighted data were corrected for geometric distortions secondary to eddy currents using a registration technique based upon the geometric model of distortions.[17]

Voxel-based diffusion data analysis

To allow voxel-based statistical comparisons, the EPI images (T2-weighted images obtained for b=0) of all subjects were spatially normalized to a customized template. This template was created by normalizing EPI images of patients and control subjects to the standard EPI template provided in SPM2 using an affine transformation with 12 degrees of freedom. The 40 EPI images were then averaged and smoothed with an 8 mm Gaussian kernel to create a study-specific template. Diffusion maps were then normalized using non-linear warp with 25 mm cutoff and 16 iterations. The FA and MD maps were then normalized using the parameters determined from the normalization of the b_0 image and smoothed with 10 mm isotropic Gaussian kernel.

Age and sex were used as confounding variables in all statistical analysis. Group comparisons were performed in SPM2 using Ancova. Multiple regressions were performed in patients for correlation of diffusion changes with the ALSFRS-R score.

For group analysis, a threshold of $p<0.05$ corrected for multiple comparisons at the voxel level was applied using False Discovery Rate. Correlations between diffusion abnormalities and the ALSFRS-R score were looked for in the regions previously found abnormal in the group comparison. For that purpose, an inclusive mask was built using the control versus patient statistical map with a statistical threshold of $p<0.05$ uncorrected. The mask was smoothed using a 5 mm Gaussian kernel. Within this mask, the clusters were considered significant at $p<0.05$ (height threshold) and corrected for multiple comparisons at the cluster level at $p<0.05$.

Voxel-based-morphometry

We determined the grey and WM volume by using the modulation step described by Good et al. 2001 [18] with some modification for SPM2 provided at http://dbm.neuro.uni-jena.de/vbm. The same parameters and thresholds as for the DTI analysis were used for image normalisation, smoothing and statistical analysis.

RESULTS (see Figure and Table)

In patients compared to controls, anisotropy was decreased bilaterally along the CST (WM underneath the precentral gyrus, the centrum semiovale and the internal capsule), in the thalamus and in the WM underneath the left insula/ventrolateral premotor cortex and the right parietal cortex. A trend was found for the right ventrolateral premotor cortex and the left parietal cortex. No increased FA was found in patients.

A bilateral and symmetric increased MD was found along the opercular regions of the frontal lobe including the ventrolateral premotor cortex, the insula and the precentral gyrus. The other regions involved were the centrum semiovale, the hippocampal formation bilaterally, and the right superior temporal gyrus. No decreased MD was found in patients.

A positive correlation between FA and the ALSFRS-R score was found bilaterally in the upper part of the CST (underneath the motor cortices and centrum semiovale), in the WM underneath the insula/ventrolateral premotor cortex, in the lateral part of the right precentral gyrus, in the cingulum, the precuneus and the splenium of the corpus callosum. No negative correlation was found.

MD abnormalities did not correlate with ALSFRS-R score.

FA correlated only negatively with disease duration in the corpus callosum and centrum semi-ovale bilaterally. MD did not correlate with disease duration.

Decreased GM volume was found bilaterally in the hippocampal formations, temporal isthmus, thalamus, inferior frontal gyrus and precentral gyrus. Other regions exhibiting a decreased GM volume were the left ventrolateral premotor cortex/insula with a trend on the right side, the left the superior temporal gyrus, left parietal and occipital cortex and right cerebellum. No GM increase was found in patients.

No abnormalities were found for maps of WM volume.

Table Brain regions exhibiting diffusion and VBM abnormalities.

Cortical area	Side	MNI coordinates x, y, z	T score	p-value
Group comparison: decreased FA				
Corticospinal tract underneath motor cortex	R	40, -24, 44	5.35	0.012
	R	36, -14, 44	4.11	0.029
	R	38, -8, 36	3.87	0.041
	L	-30, -20, 42	5.55	0.012
Corticospinal tract in centrum semiovale	L	-18, -22, 30	5.98	0.012
	L	-24, -22, 48	5.41	0.012
Corticospinal tract in centrum semiovale	R	20, -22, 42	3.94	0.037
Corticospinal tract in the internal capsule	L	-18, -20, -6	4.75	0.015
WM underneath motor cortex (medial part)	R	14, -22, 52	4.69	0.016
Thalamus		0, -18, 12	4.54	0.018
WM underneath parietal cortex	R	42, -44, 28	5.01	0.013
	L	*-44, -50, 28*	*3.70*	*0.051*
Insula	L	-42, -2, 2	4.40	0.021
WM underneath Ventrolateral premotor cortex	L	-42, -8, 18	3.85	0.042
	R	*40, 10, -2*	*3.68*	*0.052*
Group comparison: increased MD				
Precentral gyrus (Primary motor cortex)	R	48, -10, 20	6	0.023
	L	-44, -16, 20	5.21	0.029
Ventrolateral premotor cortex/Insula (post part)	R	42, 18, 2	4.28	0.048
	L	-46, 14, 0	4.64	0.042
Ventrolateral premotor cortex/Insula (ant part)	L	-34, 28, -2	4.41	0.047
Superior temporal gyrus	R	60, -26, 12	4.77	0.036
	R	50, -54, 2	4.17	0.048
Corticospinal tract in centrum semiovale	L	-24, -20, 42	4.29	0.048
	R	30, -18, 38	4.28	0.048
Hippocampal formation	R	32, -20, -14	4.01	0.048
	L	-30, -14, -18	3.92	0.049
Positive correlation between FA and the ALSFRS				
WM underneath the lateral part of the precentral gyrus	R	40, 4, 18	7.66	4568
Corticospinal tract in centrum semiovale	R	30, -8, 32	6.15	
	L	-26, -16, 38	4.87	
Corticospinal tract underneath motor cortex	L	-18, -10, 58	3.04	
	R	28, -32, 56	2.19	
Insula	L	-42, -6, 4	4.45	
	L	-30, 20, 6	3.48	
WM underneath Ventrolateral premotor cortex	R	50, 16, 4	2.29	
	L	-46, 18, -4	3.20	
Precuneus	L	-14, -60, 32	4.58	
Cingulum (posterior part)	L	-14, -42, 30	3.18	
Corpus callosum	R	10, -22, 30	4.05	

	L	-4, -16, 28	3.07	
Group comparison: decreased GM volume				
Hippocampal Formation	L	-18, -28, -10	5.04	0.034
	R	30, -34, -6	4.34	0.034
Temporal isthmus	L	-22, -52, -10	4.50	0.034
	R	28, -44, -10	4.96	0.034
Thalamus	R	6, -22, -2	4.34	0.034
	L	-12, -14, 16	3.33	0.046
Inferior Frontal Gyrus	L	-48, 16, 22	3.46	0.043
	R	44, 10, 34	3.31	0.046
Precentral gyrus (Primary motor cortex)	L	-56, -18, 28	4.52	0.034
	L	-34, -34, 58	3.71	0.037
	R	42, -24, 52	3.80	0.036
Ventrolateral premotor cortex/Insula (post part)	L	-54, -2, 4	3.71	0.037
Ventrolateral premotor cortex/Insula (ant part)	L	-48, 18, -2	4.77	0.034
	R	*50, 20, 2*	*3.18*	*0.052*
Parietal cortex	L	-34, -54, 48	3.58	0.040
Occipital lobe	L	-20, -72, -12	4.36	0.034
Cerebellum	R	10, -56, -14	3.82	0.036
Superior temporal gyrus	L	-54, -20, 12	4.92	0.034
Superior temporal sulcus	L	-52, -56, 16	3.90	0.036

Coordinates are in MNI space. Only the higher peaks per region are given. Uncorrected clusters are shown in italics.
Ant, anterior; Post, posterior; FA, fractional anisotropy; MD, mean diffusivity; GM, grey matter; WM, white matter; L, left; R, right.
ALSFRS, amyotrophic lateral sclerosis functional rating scale.

DISCUSSION

This study showed in ALS patients a reduced anisotropy along the CST correlating with the ALSFRS-R. FA was decreased into sub-cortical regions beyond the limits of the primary motor areas and correlated with the degree of motor disability. MD was increased in the opercular motor and premotor areas bilaterally and did no correlate with motor disability. Reduced GM volume in regions exhibiting MD abnormalities suggests that MD abnormalities reflected an atrophic process.

The reduced FA along the CST in ALS patients confirms previous DTI studies.[1-10] Our observation of the lack of WM volume loss confirms that anisotropy changes resulted from a loss of fibre integrity due to the axonal degeneration.[9] In previous studies, the magnitude of diffusion was reported unchanged [4,7] or increased.[1,3] These results suggest that FA is a more reliable marker of axonal degeneration than the magnitude of diffusion in ALS.

Our study confirms the correlation between the FA reduction in the CST and the functional severity of the disease, as assessed using the ALSFRS-R score, in agreement with previous studies.[1,4,8] However, correlation analysis in SPM should be cautiously interpreted and our results using the ALSFRS-R could also reflect the global progression of the disease. However, the lack of correlation with disease duration does not support this hypothesis.

Reduced FA extended largely in the sub-cortical WM far beyond the primary motor areas. Such a large extension could not be demonstrated in studies using ROI analysis which did not look for these regions.[1-8] Using whole-brain methods, no [10] or limited extra-motor abnormalities were found [9] but the first study included only seven patients [10] and the other analysed only a subvolume of the brain.[9]

We observed a bilateral increase in MD in the frontal opercular regions which correspond to regions where a cell loss was reported in vivo [19,20] and in neuropathological studies.[21]

Using VBM, we found a GM volume reduction in several of the regions exhibiting MD abnormalities. As others,[9,11,13,14] we found no hemispheric WM abnormalities along the CST. We observed a decrease in WM volume in the brain stem but an increased volume in the optic radiations and the medial prefrontal cortex possibly related to structural modifications of the frontal lobe inducing a remodelling of the sub-cortical WM.

Abnormalities outside the primary motor system are in agreement with the view that ALS is a multi-system motor degeneration.[19-21] Their correlation with the motor dysfunction suggests that these regions may also be involved in motor function.

Our VBM results suggest that MD but not FA abnormalities may be related to brain atrophy. Longitudinal studies of brain lesions using DTI would help understanding the pathogenesis of ALS and determine the potential of DTI as a marker of disease extent and severity.

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Figure Legend

Glass brain representation of the group comparison between patients with ALS and controls (clusters significant at $p<0.05$, FDR correction at the voxel level) for (A) fractional anisotropy maps showing areas of decreased anisotropy in patients, (B) mean diffusivity maps showing areas of increased diffusivity in patients, (C) grey matter volume maps showing areas of decreased volume in patients. Statistical parametric maps (D) for the positive correlation between fractional anisotropy and the ALSFRS-R score ($p<0.05$, corrected for cluster extent). The left of the images corresponds to the patient's left.