

Structural and functional brain connectome in motor neuron diseases

A multicenter MRI study

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Neurology® 2020;95:e2552-e2564. doi:10.1212/WNL.0000000000010731

Abstract

Objective

To investigate structural and functional neural organization in amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and progressive muscular atrophy (PMA).

Methods

A total of 173 patients with sporadic ALS, 38 patients with PLS, 28 patients with PMA, and 79 healthy controls were recruited from 3 Italian centers. Participants underwent clinical, neuropsychological, and brain MRI evaluations. Using graph analysis and connectomics, global and lobar topologic network properties and regional structural and functional brain connectivity were assessed. The association between structural and functional network organization and clinical and cognitive data was investigated.

Results

Compared with healthy controls, patients with ALS and patients with PLS showed altered structural global network properties, as well as local topologic alterations and decreased structural connectivity in sensorimotor, basal ganglia, frontal, and parietal areas. Patients with PMA showed preserved global structure. Patient groups did not show significant alterations of functional network topologic properties relative to controls. Increased local functional connectivity was observed in patients with ALS in the precentral, middle, and superior frontal areas, and in patients with PLS in the sensorimotor, basal ganglia, and temporal networks. In patients with ALS and patients with PLS, structural connectivity alterations correlated with motor impairment, whereas functional connectivity disruption was closely related to executive dysfunction and behavioral disturbances.

Conclusions

This multicenter study showed widespread motor and extramotor network degeneration in ALS and PLS, suggesting that graph analysis and connectomics might represent a powerful approach to detect upper motor neuron degeneration, extramotor brain changes, and network reorganization associated with the disease. Network-based advanced MRI provides an objective *in vivo* assessment of motor neuron diseases, delivering potential prognostic markers.

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Glossary

AAL = automated anatomical labeling; **ALS** = amyotrophic lateral sclerosis; **ALSFRS-r** = ALS Functional Rating Scale–revised; **DTI** = diffusion tensor MRI; **FA** = fractional anisotropy; **FTD** = frontotemporal dementia; **GM** = gray matter; **LMN** = lower motor neuron; **MMSE** = Mini-Mental State Examination; **MND** = motor neuron disease; **NBS** = network-based statistics; **PLS** = primary lateral sclerosis; **PMA** = progressive muscular atrophy; **RS fMRI** = resting-state fMRI; **UMN** = upper motor neuron; **WCFT** = Weigl Colour-Form Sorting Test; **WM** = white matter.

Motor neuron diseases (MNDs) are progressive neurodegenerative conditions characterized by breakdown of the motor system. Involvement of the upper motor neurons (UMNs) or lower motor neurons (LMNs) defines different clinical phenotypes, including amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and progressive muscular atrophy (PMA).¹ Compared with ALS, PLS and PMA are characterized by a slower rate of progression and a more benign prognosis.²

Validation of noninvasive biomarkers to characterize different MND phenotypes is a challenge of growing importance in order to recognize patients known to be at risk of more rapid progression (i.e., conversion to the ALS phenotype) prior to the appearance of clinically apparent disease. Brain MRI has been shown to have promise in detecting in vivo structural and functional brain abnormalities and in monitoring degeneration within the CNS of patients with MND.³ It is of great relevance to evaluate whether MRI biomarkers are suitable and reliable in a multicenter context.

In patients with ALS, many diffusion tensor MRI (DTI) studies have consistently identified structural alterations in a signature white matter (WM) region involving the corticospinal tract and the middle and posterior parts of the corpus callosum.⁴ DTI has proven useful in distinguishing MND variants,^{5–7} as patients with PLS showed more widespread DTI damage compared with patients with ALS,⁵ whereas the least diffuse WM damage was observed in patients with predominant LMN involvement.^{6,7}

In ALS, resting-state fMRI (RS fMRI) studies reported inconsistent results, showing either decreased or increased functional connectivity in the premotor, motor, and subcortical regions.^{8,9} To date, other MND phenotypes are yet to be explored using RS fMRI, as only one study reported increased functional connectivity within the sensorimotor, frontal, and left frontoparietal networks of patients with PLS,⁸ and no studies assessed brain functional underpinnings of PMA.

In the past decade, neuroimaging research has zeroed in on the study of changes in structural and functional connectivity at a whole-brain-system level, rather than on alterations in single brain regions,¹⁰ applying the graph theory analysis.¹¹ It has been widely demonstrated that this approach is a powerful tool to measure structural and functional reorganization in neurodegenerative

diseases,¹² including ALS.^{13,14} To date, no studies have used graph analysis and connectomics to investigate structural and functional networks in different phenotypes of MND. In addition, previous network-based studies involved single-center cohorts, thus limiting the generalizability of findings.

Considering this background, the aim of the present study was to investigate structural and functional neural organization in ALS, PLS, and PMA using graph analysis and connectomics. One of the main novelties of our study was the use of data from different centers, neuroimaging protocols, and scanners, in order to reach both reliability and reproducibility of results.

Methods

For this prospective and multicenter study, participants were recruited and evaluated clinically at 3 Italian ALS centers (San Raffaele Scientific Institute, Milan; Azienda Ospedaliera Città della Salute e della Scienza, Turin; and Università degli Studi della Campania “Luigi Vanvitelli,” Naples) from 2009 to 2017 in the framework of a large, observational study. MRI scans were obtained from all participants using two 3T scanners: Philips Medical Systems [Best, the Netherlands] Intera machine (Milan and Turin) and GE [Chicago, IL] Signa HDxt machine (Naples). All MRI data were analyzed at the Neuroimaging Research Unit, Division of Neuroscience, San Raffaele Scientific Institute and Vita-Salute San Raffaele University, Milan, Italy.

Participants

A total of 239 patients with sporadic MND (173 with classic ALS, 38 with PLS, and 28 with PMA) were consecutively recruited from those routinely evaluated at the 3 clinical centers (table 1). Patients with classic ALS (131 from Milan/Turin and 42 from Naples) met a diagnosis of probable or definite ALS according to the revised El Escorial criteria.¹⁵ Thirty-eight patients (all from the Milan/Turin dataset) were diagnosed with PLS according to Pringle criteria at the last available clinical follow-up.¹⁶ Twenty-eight patients had PMA (all from the Milan/Turin dataset).¹⁷ All patients were receiving riluzole at study entry. Seventy-nine age- and sex-matched healthy controls (61 from Milan/Turin and 18 from Naples) were recruited by word of mouth (table 1), based on the following criteria: normal neurologic assessment; Mini-Mental State Examination (MMSE) score ≥ 28 , and no family history of neurodegenerative diseases. Exclusion criteria for all participants (patients and healthy controls) were medical

Table 1 Demographic and clinical features of patients with amyotrophic lateral sclerosis (ALS), patients with primary lateral sclerosis (PLS), and patients with progressive muscular atrophy (PMA) and matched healthy controls (HC)

	HC	ALS	PLS	PMA	<i>p</i> , ALS vs HC	<i>p</i> , PLS vs HC	<i>p</i> , PMA vs HC	<i>p</i> , ALS vs PLS	<i>p</i> , ALS vs PMA	<i>p</i> , PLS vs PMA
N	79	173	38	28						
Age, y	61.84 ± 8.82 (42.00–81.81)	61.56 ± 10.64 (28.47–86.12)	63.20 ± 7.89 (43.87–80.26)	58.44 ± 8.99 (39.62–73.91)	1.00	1.00	0.69	1.00	0.71	0.31
Women/men	46/33	72/101	20/18	8/20	0.02	0.69	0.01	0.28	0.22	0.08
Education, y	12.87 ± 4.38 (5–24)	10.41 ± 4.42 (3–24)	10.40 ± 4.43 (2–18)	10.82 ± 4.81 (5–24)	<0.001	0.03	0.23	1.00	1.00	1.00
Onset, limb/bulbar	—	128/45	33/5	27/1	—	—	—	0.10	0.01	0.23
Disease duration, mo	—	18.97 ± 17.66 (2–136)	79.32 ± 60.46 (8–247)	69.14 ± 98.61 (4–457)	—	—	—	<0.001	<0.001	1.00
ALSFRS-r, 0–48	—	37.92 ± 6.95 (11–47)	37.16 ± 5.72 (22–44)	40.14 ± 6.00 (25–48)	—	—	—	1.00	0.31	0.22
UMN score	—	9.82 ± 4.75 (0–16)	13.67 ± 2.11 (10–16)	2.14 ± 1.70 (0–5)	—	—	—	0.001	<0.001	<0.001
MRC global score	—	96.04 ± 23.86 (5–148)	112.86 ± 10.43 (80–121)	96.25 ± 17.73 (51–119)	—	—	—	0.004	1.00	0.04
Disease progression rate	—	0.78 ± 0.70 (0.04–4.11)	0.31 ± 0.49 (0.03–2.89)	0.33 ± 0.42 (0–2.00)	—	—	—	<0.001	0.002	1.00

Abbreviations: ALSFRS-r = Amyotrophic Lateral Sclerosis Functional Rating Scale–revised; MRC = Medical Research Council; UMN = upper motor neuron. Values are numbers or means ± SD (range). Disease duration was defined as months from onset to date of MRI scan. *p* Values refer to analysis of variance models, followed by post hoc pairwise comparisons (Bonferroni-corrected for multiple comparisons), or χ^2 test.

illnesses or substance abuse that could interfere with cognitive functioning; any (other) major systemic, psychiatric, or neurologic diseases; other causes of brain damage, including lacunae; and extensive cerebrovascular disorders at MRI.

Disease severity was assessed using the ALS Functional Rating Scale–revised (ALSFRS-r).¹⁸ The baseline rate of disease progression was defined according to the following formula: (48 – ALSFRS-r score)/time between symptom onset and first visit. Muscular strength was assessed by manual muscle testing based on the Medical Research Council scale, and clinical UMN involvement was graded by totaling the number of pathologic UMN signs on examination.¹⁹ For the UMN score, we also considered the presence of nondefinite UMN signs such as reduced but still evocable reflexes in muscles with LMN signs, which were detected in few individuals with PMA.

Standard protocol approvals, registrations, and patient consents

The local ethical standards committee on human experimentation approved the study protocol and all participants provided written informed consent (ethical committee numbers RF-2011-02351193 and ConnectALS).

Neuropsychological assessment

Neuropsychological assessments were performed by experienced neuropsychologists unaware of the MRI results (table

e-1, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). The following cognitive functions were evaluated: global cognitive functioning with the MMSE²⁰; long- and short-term verbal memory with the Rey Auditory Verbal Learning Test²¹ and the digit span forward,²² respectively; executive functions with the digit span backward,²³ the Stroop interference test,²⁴ the Cognitive Estimation Task,²⁵ the Weigl Colour-Form Sorting Test (WCFT),²⁶ the Wisconsin Card Sorting Test,²⁷ or the Modified Card Sorting Test,²⁸ and Raven Coloured Progressive Matrices²⁹; fluency with phonemic and semantic fluency tests^{21,26,30} and relative fluency indices (controlling for individual motor disabilities)³¹; and language with the Italian battery for the assessment of aphasic disorders.³² Mood was evaluated with the Hamilton Depression Rating Scale³³ or Beck Depression Inventory.³⁴ The presence of behavioral disturbances was assessed with the Frontal Behavioral Inventory³⁵ and the Amyotrophic Lateral Sclerosis–Frontotemporal Dementia Questionnaire³⁶ administered to patients' caregivers. Healthy controls underwent the entire assessment except for the Stroop interference test, the Cognitive Estimation Task, and the WCFT.

Cognitive test scores were not available for all patients. Table e-1 (data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>) reports the number of patients who performed each cognitive test.

MRI analysis

Using two 3T MRI scanners, T1-weighted, T2-weighted, fluid-attenuated inversion recovery, DTI, and RS fMRI sequences were obtained from all participants (table e-2, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3> for MRI sequence parameters). An experienced observer, blinded to participants' identity and diagnosis, performed MRI analysis. Gray matter (GM) was parcellated into 220 similarly sized brain regions, which included cerebral cortex and basal ganglia but excluded the cerebellum (figure 1, Ia).³⁷ DTI and RS fMRI preprocessing and construction of brain structural and functional connectome have been described previously³⁸ (figure 1, Ia and Ib).

Global brain and lobar network analysis

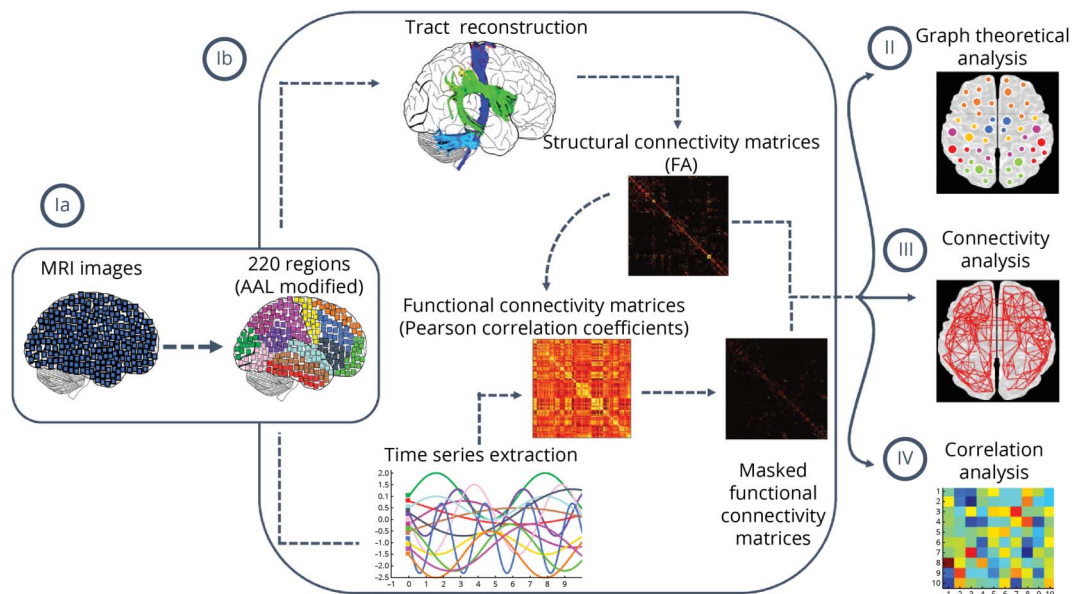
Global and mean lobar structural and functional network characteristics were explored using the Brain Connectivity MATLAB toolbox (brain-connectivity-toolbox.net). Network metrics, including nodal strength, characteristic path length, local efficiency, and clustering coefficient were assessed to characterize the topologic organization of global brain and lobar networks in patients and healthy controls (figure 1, II).³⁹ In order to investigate the network characteristics in different areas of the brain, the 220 regions of interest from both hemispheres were grouped into 6 anatomical macroareas (hereafter referred to as brain lobes): temporal, parietal, occipital, frontoinsular, basal ganglia, and sensorimotor areas.³⁷ Structural network properties were generated according to fractional anisotropy (FA) values, while analysis of brain network function was based on functional connectivity strength values (*z*-transformed Pearson correlation coefficients).

Global and lobar metrics were compared between groups using age-, sex-, and MRI scanner–adjusted analysis of variance models, followed by post hoc pairwise comparisons, Bonferroni-corrected for multiple comparisons ($p < 0.05$, SPSS Statistics 22.0 [SPSS Inc., Chicago, IL]). In addition, to evaluate the effects of the patients with full-blown dementia into the results, the analyses also were performed without the 8 patients with ALS–frontotemporal dementia (FTD). Furthermore, comparison between patients with ALS and controls, recruited only from Milan or Turin centers, was performed in order to assess the reproducibility of the findings when MRIs were obtained using a single MRI scanner.

Connectivity analysis

Network-based statistics (NBS)⁴⁰ were performed to assess regional FA and functional connectivity strength network data in patients and controls at the level of significance $p < 0.05$ (figure 1, III). The largest (or principal) connected component and the smaller clusters of altered connections, which were not included in the principal component, were studied.^{40,41} A corrected *p* value in the direct comparison between patients with ALS and healthy controls (both provided by Milan or Turin and Naples centers) was calculated for each component using an age-, sex-, and MRI scanner–adjusted permutation analysis (10,000 permutations). Regarding the other comparisons, only patients with MND and controls from Milan or Turin centers were included in the age- and sex-adjusted permutation analysis. In line with previous global and lobar network analysis, NBS was performed also excluding patients with ALS-FTD and patients with ALS or controls recruited at the Naples center.

Figure 1 MRI processing pipeline



(Ia) Gray matter was parcellated in 220 similarly sized brain regions, which included cerebral cortex and basal ganglia but excluded the cerebellum. (Ib) Diagram reports diffusion tensor MRI and resting-state fMRI preprocessing steps and construction of brain structural and functional connectomes. Structural and functional matrices were the input for 3 distinctive analyses: (II) global and lobar graph analysis, (III) connectivity analysis, and (IV) correlation analysis. AAL = automated anatomical labeling; FA = fractional anisotropy.

Figure 2 Summary of altered structural and functional metrics in the different motor neuron disease variants

		Structural connectivity (FA)																																															
ALS	Global brain analysis	Whole brain																																															
	Lobar network analysis	Fronto-insular					Temporal					Parietal					Occipital					Basal ganglia					Sensorimotor																						
	Connectivity analysis	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S						
	Functional connectivity																																																
	Global brain analysis	Whole brain																																															
	Lobar network analysis	Fronto-insular					Temporal					Parietal					Occipital					Basal ganglia					Sensorimotor																						
Connectivity analysis	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	
PLS	Structural connectivity (FA)																																																
	Global brain analysis	Whole brain																																															
	Lobar network analysis	Fronto-insular					Temporal					Parietal					Occipital					Basal ganglia					Sensorimotor																						
	Connectivity analysis	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S
	Functional connectivity																																																
	Global brain analysis	Whole brain																																															
Lobar network analysis	Fronto-insular					Temporal					Parietal					Occipital					Basal ganglia					Sensorimotor																							
Connectivity analysis	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	FI	T	P	O	BG	S	

Three shades of green are used to define the severity of damage in terms of percentage of altered metrics (global and lobar analyses) and percentage of altered connections between 2 lobes (connectivity analysis). The 3 shades of green depict the following ranks: 1%–25% (light green), 26%–50% (medium green), and 51%–75% (dark green). White background represents the absence of alterations. ALS = amyotrophic lateral sclerosis; BG = basal ganglia; FA = fractional anisotropy; FI = fronto-insular; O = occipital; P = parietal; PLS = primary lateral sclerosis; S = sensorimotor; T = temporal.

Correlation analysis

To assess the relationship between structural and functional brain network properties and clinical and neuropsychological variables, correlation analysis was performed in each patient group. Partial correlations between MRI measures (exhibiting significant differences between patients and controls), clinical variables, and cognitive data were estimated using Pearson correlation coefficient (R), at the level of significance $p < 0.05$ (figure 1, IV). Correlation analyses were adjusted for age, sex (in patients with PLS), and age, sex, and MRI scanner (in patients with ALS). Relationship with neuropsychological data was also adjusted for education and ALSFRS-r. Correlation analyses at global or lobar and regional level were also controlled for multiple comparisons, applying respectively Bonferroni and false discovery rate adjustment.

Data availability

The dataset used and analyzed in this study will be made available by the corresponding author upon request to qualified researchers (i.e., affiliated with a university or research

institution or hospital). Additional tables and figures are available from Dryad (tables e-1–e-7 and figures e-1 and e-2, <https://doi.org/10.5061/dryad.v15dv41t3>).

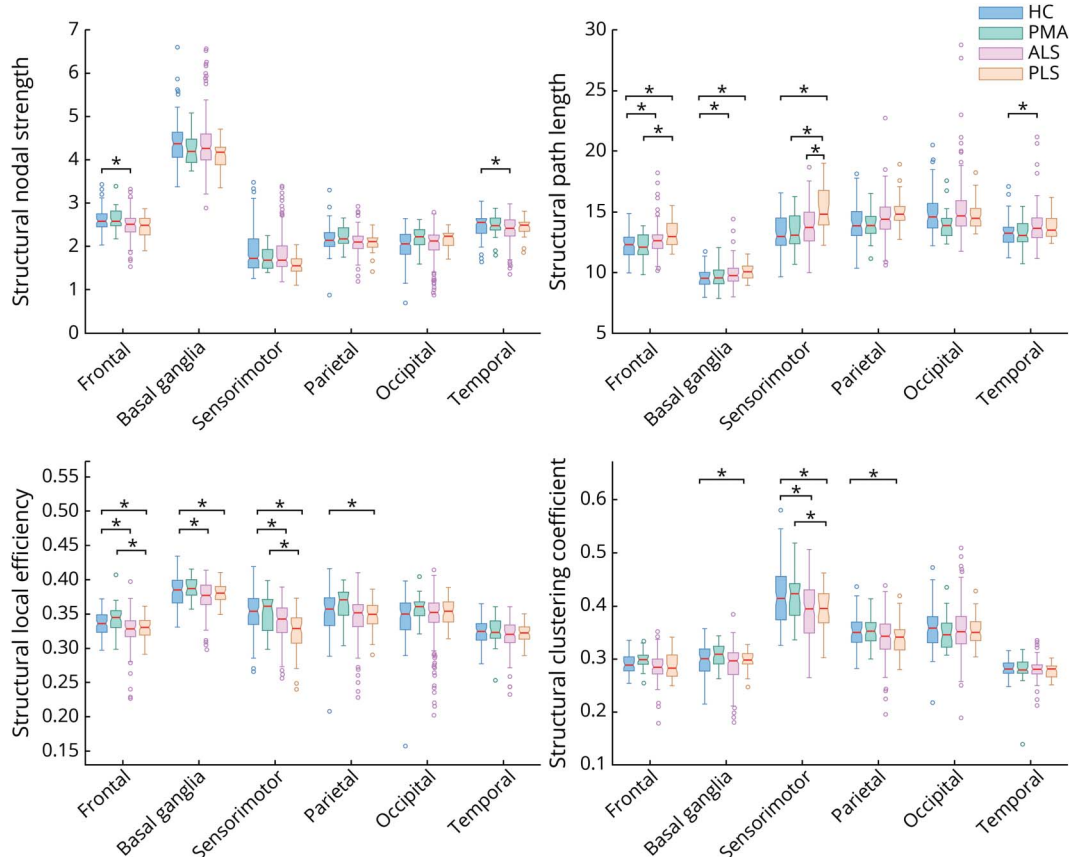
Results

A summary of structural and functional altered metrics at global, lobar, and regional levels in the different MND phenotypes is shown in figure 2.

Patients with ALS vs healthy controls

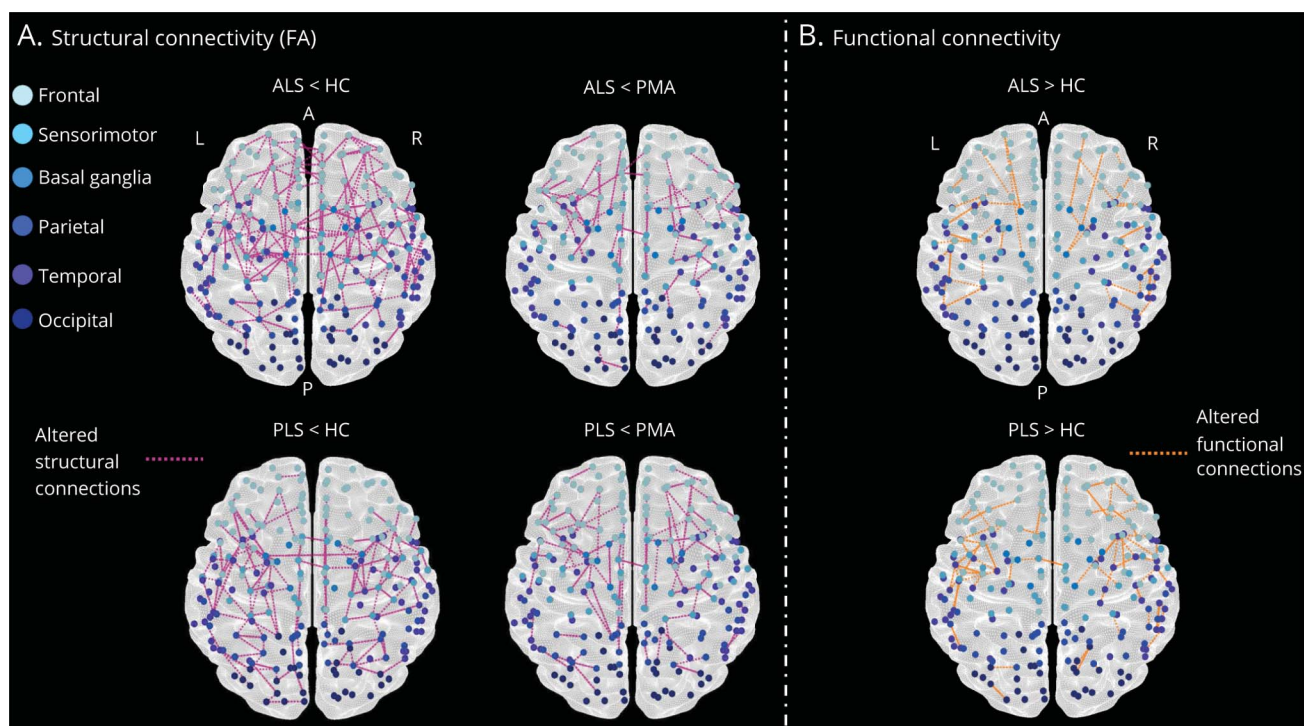
Compared with healthy controls, patients with ALS showed altered structural global network properties (lower mean local efficiency) (table e-3, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Patients with ALS showed a reduced mean structural local efficiency in the sensorimotor, basal ganglia, and frontal networks and longer path length in basal ganglia, frontal, and temporal networks relative to healthy controls (figure 3 and table e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). They showed also reduced mean nodal

Figure 3 Graph analysis properties of brain lobar networks in patients with amyotrophic lateral sclerosis (ALS), patients with primary lateral sclerosis (PLS), and patients with progressive muscular atrophy (PMA) and healthy controls (HC)



Boxplot of structural nodal strength, path length, local efficiency, and clustering coefficient of each brain lobe are shown for patient groups and matched HC. The red horizontal line in each box plot represents the median, the 2 lines just above and below the median represent the 25th and 75th percentiles, whiskers represent the minimum and maximum values, and all the dots outside the confidence interval are considered as outliers. * $p < 0.05$. All the comparisons were adjusted for age, sex, and MRI scanner. ALS = amyotrophic lateral sclerosis; PLS = primary lateral sclerosis; PMA = progressive muscular atrophy.

Figure 4 Subnetworks showing altered structural and functional connectivity in patients with amyotrophic lateral sclerosis (ALS), patients with primary lateral sclerosis (PLS), and patients with progressive muscular atrophy (PMA) relative to healthy controls (HC) and between patient groups



Altered structural (A) and functional (B) connections are represented in magenta and orange, respectively. All the comparisons were adjusted for age, sex, and MRI scanner. Six shades of blue were used to define the belonging of each node to different lobes starting with light blue (frontal lobe) to dark blue (posterior lobe, i.e., occipital). A = anterior; FA = fractional anisotropy; P = posterior.

strength in frontal and temporal regions relative to controls (figure 3 and table e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Patients with ALS had preserved global and lobar functional nodal properties compared with controls (tables e-3 and e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). NBS showed structural changes in patients with ALS relative to controls: decreased FA in the sensorimotor networks, including precentral and postcentral gyri, supplementary motor area, and basal ganglia, and among the connections of the medial and lateral prefrontal cortex (figure 4A). Patients with ALS also showed increased functional connectivity compared with controls involving precentral gyrus and middle and superior frontal gyri (figure 4B). The listed results were confirmed excluding from the analysis patients with ALS-FTD (table e-5 and figure e-1, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>) or patients with ALS and healthy controls acquired at the Naples center (table e-6 and figure e-2, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>).

Patients with PLS vs healthy controls

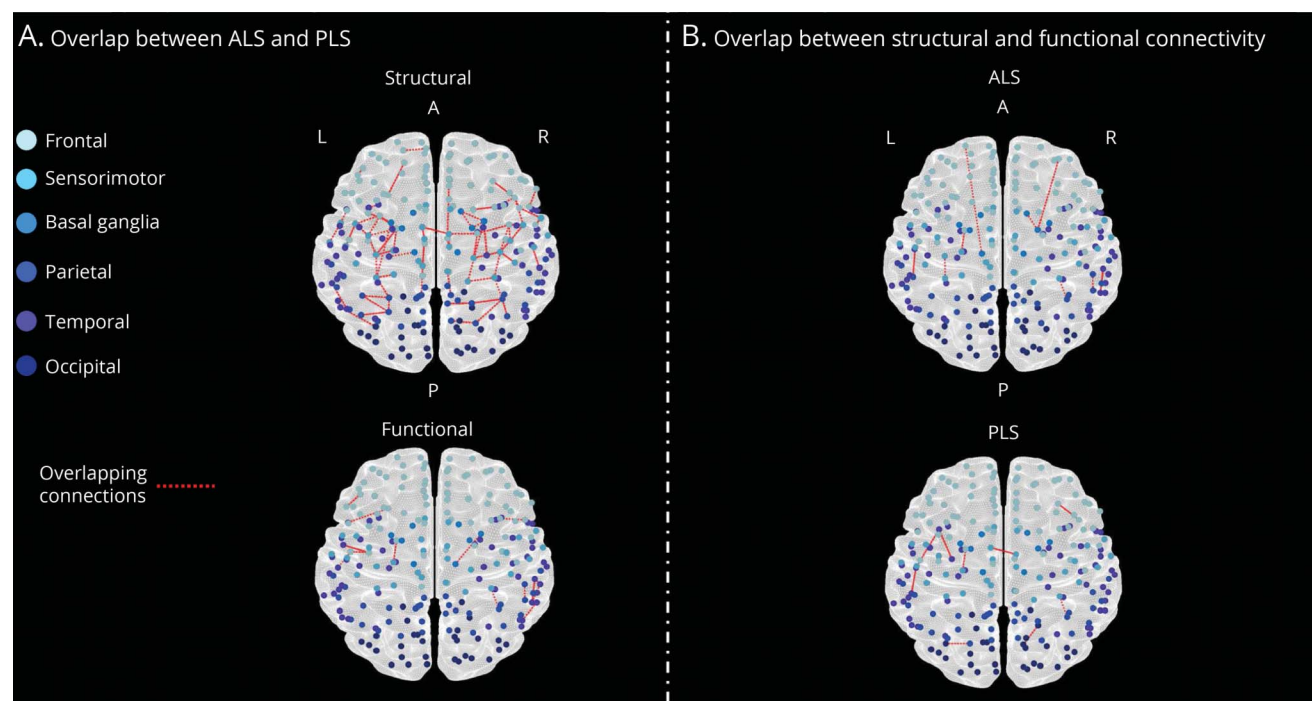
Compared with healthy controls, patients with PLS showed altered structural global network properties (lower mean local efficiency and clustering coefficient, longer mean path length) (table e-3, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>).

5061/dryad.v15dv41t3). Patients with PLS showed a reduced mean structural local efficiency and clustering coefficient and longer path length in the sensorimotor, basal ganglia, frontal, and parietal areas relative to healthy controls (figure 3 and table e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Patients with PLS had relatively preserved global and lobar functional nodal properties compared with controls (figure 3 and tables e-3 and e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Using NBS, widespread structural changes were observed in patients with PLS relative to controls: decreased FA within the sensorimotor networks, including precentral and postcentral gyri, supplementary motor area, and basal ganglia, and among connections within temporal and occipito-parietal areas (figure 4A). NBS analysis showed that patients with PLS had higher functional connectivity in the sensorimotor, basal ganglia, and temporal networks relative to controls (figure 4B). PLS structural and functional damage mimics that observed in classical ALS (figure 5).

Patients with PMA vs healthy controls

Patients with PMA did not show differences in structural or functional graph and connectivity properties at the global or regional level (figures 3 and 4 and tables e-3 and e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>).

Figure 5 Subnetworks showing overlapping affected connections



Overlapping affected connections in ALS and patients with PLS in structural and functional MRI (A) and overlapping structural and functional affected connections within the two groups (B) are represented in red. Six shades of blue were used to define the belonging of each node to different lobes starting with light blue (frontal lobe) to dark blue (posterior lobe, i.e., occipital). A = anterior; ALS = amyotrophic lateral sclerosis; FA = fractional anisotropy; L = left; P = posterior; PLS = primary lateral sclerosis; R = right.

Patients with ALS vs patients with PLS

Patients with ALS and patients with PLS did not show differences in structural and functional graph properties at global level (table e-3, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Patients with PLS demonstrated altered local structural, but not functional, alterations in sensorimotor network relative to ALS group (longer path length) (figure 3 and table e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). NBS did not show differences between patients with ALS and patients with PLS (figure 4). These findings were confirmed excluding patients with ALS-FTD from the analysis (table e-5 and figure e-1, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>).

Patients with ALS vs patients with PMA

Patients with ALS and patients with PMA did not show differences in structural and functional graph properties at both global and lobar level (figure 3 and tables e-3 and e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). However, patients with ALS showed decreased FA relative to patients with PMA within the sensorimotor network including precentral and postcentral gyri and frontal network (figure 4A). NBS did not show functional connectivity differences between patients with ALS and patients with PMA (figure 4B). The presented results have been validated excluding patients with ALS-FTD from the analysis (table e-5 and figure e-1, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>).

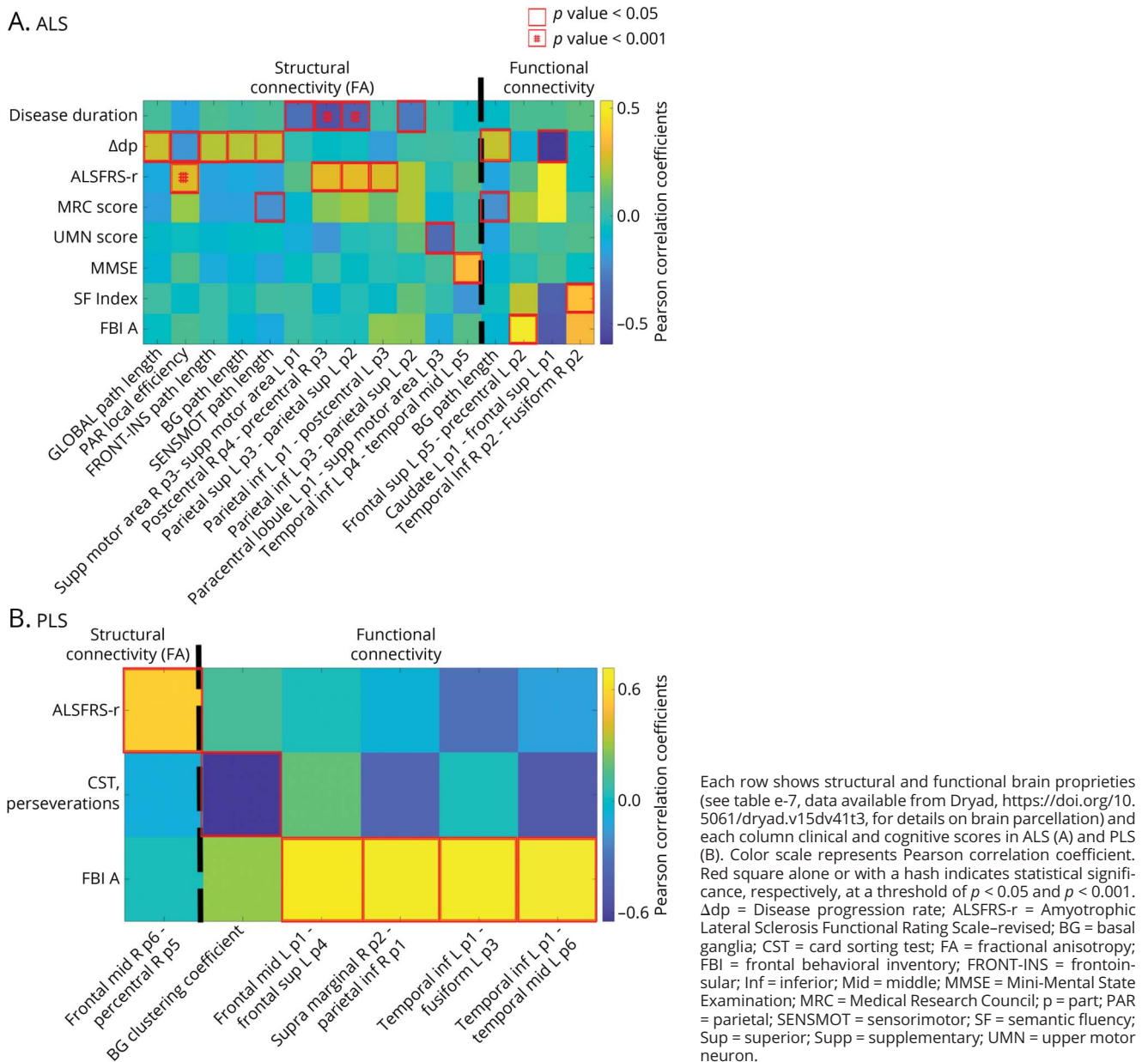
Patients with PLS vs patients with PMA

Patients with PLS and patients with PMA did not show differences in structural and functional graph properties at a global level (table e-3, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). Patients with PLS demonstrated altered local structural, but not functional, alterations in sensorimotor and frontal networks relative to the PMA group (lower mean local efficiency and clustering coefficient and longer mean path length) (figure 3 and table e-4, data available from Dryad, <https://doi.org/10.5061/dryad.v15dv41t3>). In the NBS analysis, patients with PLS showed decreased FA relative to patients with PMA in the connections within and among sensorimotor network basal ganglia, frontal, and parieto-occipital areas (figure 4A). NBS did not show functional connectivity differences between patients with PLS and patients with PMA (figure 4B).

Correlation analysis

In patients with ALS, graph analysis structural brain changes mostly correlated with clinical disease severity (figure 6A). Indeed, a longer path length was related to disease progression rate (Δdp) both at the global ($R = 0.25, p = 0.01$) and lobar levels, particularly within sensorimotor ($R = 0.23, p = 0.01$), basal ganglia ($R = 0.22, p = 0.02$), and frontal-insular ($R = 0.22, p = 0.02$) networks. Moreover, structural local efficiency in the parietal network correlated negatively with Δdp ($R = -0.20, p = 0.03$) and positively with ALSFRS-r score ($R = 0.29, p < 0.001$).

Figure 6 Characterization of the relationship between structural and functional MRI metrics and clinical/cognitive data in patients with amyotrophic lateral sclerosis (ALS) and patients with primary lateral sclerosis (PLS)



Regarding regional analysis, in patients with ALS, a decreased FA of the connections within the temporal network correlated with a worse performance in global cognition ($R = 0.36$, $p = 0.03$), while a higher disruption within the sensorimotor areas correlated with longer disease duration (R ranging from -0.51 to -0.28 , $p < 0.05$) and greater disease severity (R ranging from -0.34 to 0.30 , $p < 0.05$). In patients with ALS, functional connectivity changes within basal ganglia network and connections between basal ganglia and premotor areas correlated with disease progression (R ranging from -0.60 to 0.24 , $p < 0.05$). Moreover, higher functional connectivity, within extramotor areas (temporo-frontal network), correlated with worse performance at executive ($R = 0.37$, $p = 0.01$) and behavioral

tests ($R = 0.50$, $p = 0.04$). In patients with PLS, disrupted structural connections within motor and premotor areas correlated with lower ALSFRS-r scores ($R = 0.56$, $p = 0.02$) (figure 6B). On the other hand, functional connectivity alterations were more related to cognitive performance. Particularly, functional clustering coefficient correlated with executive dysfunction within the basal ganglia network ($R = -0.65$, $p = 0.04$). Moreover, higher functional connectivity of the connections among temporal and frontal areas correlated with a worse performance on behavioral testing (R ranging from 0.65 to 0.72 , $p < 0.05$). Correlations were not assessed in the PMA group because no significant structural and functional differences were found in the previous analyses relative to controls.

Discussion

Using graph analysis and connectomics to explore structural and functional brain networks, the present multicenter study showed that clinical variants within the MND spectrum result in different patterns of brain network changes. Patients with ALS showed altered structural global and lobar network properties and regional connectivity, with a specific involvement of sensorimotor, basal ganglia, frontal, and temporal areas. The structural damage in the PLS group was found in the sensorimotor network, together with more widespread damage in extramotor regions, such as the parietal lobe. On the contrary, patients with PMA showed preserved structural and functional connectomes. Finally, in both ALS and PLS groups, alterations in structural connectivity correlated with measures of motor impairment, while functional connectivity disruptions were mostly related to executive dysfunctions and behavioral disturbances. These results proved to be independent of the presence of full-blown dementia, being confirmed also excluding 8 patients with ALS-FTD from the analyses.

To date, several MRI studies have highlighted structural^{4,6} or functional^{8,9} “signatures” of different phenotypes within the MND spectrum. However, while DTI studies have described consistent results, the literature of functional studies has reported inconsistent findings. Moreover, the above-mentioned studies have zeroed in on the study of structural and functional alterations at a voxel or regional level, rather than on alterations at brain-system level.¹⁰ In order to overcome this limitation, the present study has applied advanced network-based neuroimaging techniques, aiming to provide information about how networks are embedded and interact in the brain of different phenotypes within the MND spectrum, deepening previous findings of standard MRI techniques. Whereas whole-brain approaches might detect alterations at a voxel or regional level, connectome analysis considers the relationships between degenerating connections and is able to provide connectivity information about the integrated nature of brain.⁴² Another advantage of this new approach is that it may help in bridging the gap between different types of data, such as anatomical and functional connectivity. In fact, the use of a common parcellating system and the same statistical approach allows a straightforward comparison between the 2 types of information.

Graph analysis and connectomics have been applied to characterize structural and functional damage in patients with ALS. Particularly, our findings are consistent with previous DTI studies that reported the presence of an impaired sub-network including bilateral primary motor regions, supplementary motor areas, and basal ganglia.⁴³ Furthermore, our study highlights that affected extramotor regions are structurally connected to the sensorimotor network, known to be the epicenter of the degenerative process of the disease.⁴⁴ This hypothesis is consistent with the pattern of progression of TDP-43 pathologic burden described by Brettschneider

et al.⁴⁴ in postmortem tissue, and supports a network-based degeneration model in ALS,⁴⁵ although longitudinal MRI studies are needed to validate this hypothesis.

On the other hand, very few RS fMRI studies applied network-based analyses on patients with ALS, demonstrating complex connectivity alterations encompassing frontal, temporal, occipital, and subcortical regions.^{14,46} In our study, we found increased functional connectivity in sensorimotor, basal ganglia, and frontal areas in patients with ALS. Our results are mostly consistent with previous studies, although showing more focal functional rearrangements, possibly due to differences in disease stage and methodology (as in our study only functional edges with existing structural connections were considered). Although our study confirms previous findings, our strength is the application of advanced neuroimaging techniques in an unprecedented number of patients with ALS due to the fact that is a multicenter study. In light of this, the large number of patients has a strong impact on the statistical power of the analysis and influenced the quality and reliability of our results. This is the first study that applied graph theory in patients with PLS and patients with PMA. Particularly patients with PLS showed widespread structural and functional alterations encompassing both motor and extramotor areas with a pattern resembling classic ALS (figure 5), in line with previous studies.^{5,47} By contrast, patients with PMA did not show any structural or functional damage relative to healthy controls. These findings are in line with previous studies that could not demonstrate CNS damage in patients with PMA,^{6,7} even using a technique that is highly sensitive to local disruptions in the brain networks. We have demonstrated the high sensitivity of graph-based analysis to detect different disease-related disconnection patterns and its potential use to facilitate clinical diagnosis and offer new insights into syndromes’ clinical diversity.

Patients with ALS and patients with PLS are characterized by more widespread structural than functional damage relative to healthy controls (figure 4). The presence of functionally unaffected but structurally impaired nodes and connections in both groups suggests that structural alterations may be earlier in the course of the disease compared with functional network abnormalities. In keeping with the network-based hypothesis,⁴⁸ pathologic alterations physically spread along neuroanatomical connections in the brain; therefore, it is reasonable to speculate that functional connectivity alterations may follow the structural disruption of the brain network. These findings are also in line with those recently observed in other neurodegenerative diseases.³⁸ However, it should also be considered that this cross-sectional study cannot fully address the temporal sequence or causal relationships between structural and functional abnormalities and different techniques (i.e., DTI and RS fMRI) may intrinsically show different sensitivities to underlying biological processes.

In the present study, the regional (i.e., NBS) analysis showed greater sensitivity for the detection of structural and,

particularly, functional damage of brain networks, compared with the evaluation of single network properties. Moreover, the results of the global/lobar structural analysis provided some apparent inconsistencies across different network measures. For example, although structural nodal strength did not show significant alterations in the sensorimotor regions of patients with MND compared with healthy controls, all other graph theoretical measures (i.e., local efficiency, clustering coefficient, and path length) did. Given the interdependence of these measures, and the fact that nodal strength was on average lower than in healthy controls in all MND groups, we argue that nodal strength might simply be less sensitive than other measures to the structural disruption of the sensorimotor network in our cohort. This might differ in other anatomical areas with different topologic organization, such as the temporal regions (which are also affected in MND¹³), where nodal strength and path length were significantly altered in patients with ALS, in contrast with the sparing of other network properties. Therefore, our results support the utility of graph theoretical measures used in combination, rather than as single measures, also considering the current impossibility of establishing a clear-cut neuropathologic substrate for each of these.

Concerning the correlation analysis, our findings suggest that the presence of structural damage in patients with ALS in motor, premotor, and parietal regions, key elements for the correct programming and processing/execution of the movement, is specifically related to clinical measures of motor impairment, rate of progression, and disease duration. Particularly, the rate of progression was more closely related to global and lobar alterations, while measures of disease severity and duration were associated with regional connectivity disruption, although correlation coefficients were generally moderate in size (0.2–0.4). By contrast, the (possibly maladaptive) increase of functional connectivity in frontal and temporal regions was related to executive dysfunctions and behavioral impairment, as previously shown.⁸ Patients with PLS showed a similar pattern of correlations, although with a lower number of significant findings, partly due to the small sample size. Nevertheless, a strong relationship between functional connectivity in extramotor areas and behavioral impairment was found, to point out that the cognitive profile in patients with PLS traced the one in patients with ALS, with more prominent deficits in the behavioral domain.

One of the most important caveats of previous studies is the single-center origin of imaging data that limits the generalizability of findings. In light of this, one of the main novelties of our study was including data from different centers, neuroimaging protocols, and scanners. Although MRI protocols were not harmonized between the 2 acquisition centers, the obtained results proved to be solid (as shown by the single-center subanalysis) and the approach was easily reproducible despite protocol differences. On the other hand, this study is not without limitations. First, the PLS and PMA groups were

relatively small, affecting the statistical power of the results. In particular, the absence of differences between patients with PMA and healthy controls might partially depend on the relatively small sample size. However, patients with PMA also showed significant structural sparing compared with both patients with ALS and patients with PLS, consistent with previous studies performed using different techniques,^{6,7} as well as with the common notion of PMA as a predominant lower motor neuron disease. Second, cognitive test scores were not available for all patients. However, we selected tests for which patient samples were sufficiently represented. Third, healthy controls showed higher education than patients with ALS and patients with PLS, although the analyses involving neuropsychological data were adjusted for education. Fourth, we chose arbitrarily to parcellate the brain into 220 similarly sized regions based on the automated anatomical labeling (AAL) atlas, excluding the cerebellum. Technically, network science applied to the human brain has yet to reach consensus regarding the best way to divide the brain into its most relevant anatomical units⁴⁹ as well as to threshold connectivity matrices.⁵⁰ The definition of an optimal framework has not yet been reached in the neuroscience community, and the field of network data analysis remains an area of active methodologic development. However, it is generally acknowledged that similarly sized regions of interest avoid larger regions to have higher connectivity because of their larger surface. The exclusion of the cerebellum was motivated by the fact that the AAL atlas is rather inaccurate to segment this anatomical region, and other, unbiased ad hoc methods should be preferred in future studies. Fifth, although RS fMRI data were carefully registered to and masked with GM maps to avoid a regional atrophy influence, a possible partial volume effect on our results cannot be excluded. Finally, this is a cross-sectional study. Longitudinal studies are needed to evaluate structural and functional changes along with the disease progression over time and are warranted in order to confirm the role of MRI network-based analysis to examine differential diagnosis and prognosis of MND in a clinical context, as well to support the hypothesis of a single continuum from ALS to FTD.

This study showed considerable motor and extramotor network degeneration in patients with ALS and even more widespread damage in patients with PLS, suggesting that graph analysis and connectomics might represent a powerful approach to detect overlapping and specific regions of damage in different MND phenotypes. Importantly, these techniques have proven robust and suitable to manage the multicenter setting variability. Network-based advanced MRI analyses hold promise to provide an objective in vivo assessment of MND-related pathologic changes, delivering potential prognostic markers.

Study funding

This study was partially supported by the Italian Ministry of Health (grant RF-2011-02351193) and AriSLA-Fondazione Italiana di Ricerca per la SLA (project ConnectALS).

Disclosures

S. Basaia, C. Cividini, F. Trojsi, N. Riva, C. Femiano, E.G. Spinelli, C. Moglia, V. Castelnovo, Y. Falzone, M.R. Monsurrò, A. Falini report no disclosures. F. Agosta is Section Editor of *NeuroImage: Clinical*; has received speaker honoraria from Novartis, Biogen Idec, and Philips; and receives or has received research support from the Italian Ministry of Health, AriSLA (Fondazione Italiana di Ricerca per la SLA), and the European Research Council. E. Canu has received research support from the Italian Ministry of Health. A. Chiò is Editor of *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* and *Neurologic Sciences*; received compensation for consulting services and/or speaking activities from Biogen Idec, Italfarmaco, Roche, Cytokinetics, and Avenir; and receives or has received research support from the Italian Ministry of Health and the Italian Ministry of Education, University and Research. G. Tedeschi received compensation for consulting services and/or speaking activities from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries, AbbVie, Allergan, and Roche; and receives research support from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries, Roche, Italian Ministry of Health, Allergan, AbbVie, and Mylan. M. Filippi is Editor-in-Chief of the *Journal of Neurology*; received compensation for consulting services and/or speaking activities from Bayer, Biogen Idec, Merck-Serono, Novartis, Roche, Sanofi Genzyme, Takeda, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARI SLA (Fondazione Italiana di Ricerca per la SLA). Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* January 8, 2020. Accepted in final form June 3, 2020.

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Federica Agosta, MD, PhD	Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, and Vita-Salute San Raffaele University, Milan, Italy	Design and conceptualization of study, interpretation of data, drafted and revised the manuscript for intellectual content, obtained funding

Appendix (continued)

Name	Location	Contribution
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Continued

Appendix (continued)

Name	Location	Contribution
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Massimo Filippi, MD	Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, Neurology Unit, Neurophysiology Unit, IRCCS San Raffaele Scientific Institute, and Vita-Salute San Raffaele University, Milan, Italy	Design and conceptualization of study, interpretation of data, drafted and revised the manuscript for intellectual content, obtained funding

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Structural and functional brain connectome in motor neuron diseases: A multicenter MRI study

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Neurology 2020;95:e2552-e2564 Published Online before print September 10, 2020

DOI 10.1212/WNL.0000000000010731

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