




# Amyotrophic lateral sclerosis phenotypes significantly differ in terms of magnetic susceptibility properties of the precentral cortex

Giorgio Conte<sup>1</sup> · Valeria Elisa Contarino<sup>1</sup> · Silvia Casale<sup>1</sup>  · Claudia Morelli<sup>2</sup> · Sara Sbaraini<sup>3</sup> · Elisa Scola<sup>1</sup> · Francesca Trogu<sup>2,4</sup> · Silvia Siggillino<sup>1</sup> · Claudia Maria Cinnante<sup>1</sup> · Luca Caschera<sup>1</sup> · Francesco Maria Lo Russo<sup>1</sup> · Fabio Maria Triulzi<sup>1,4</sup> · Vincenzo Silani<sup>2,4</sup>

Received: 18 June 2020 / Revised: 27 October 2020 / Accepted: 19 November 2020

© European Society of Radiology 2021

## Abstract

**Objectives** The aim of our study was to investigate whether the magnetic susceptibility varies according to the amyotrophic lateral sclerosis (ALS) phenotypes based on the predominance of upper motor neuron (UMN)/lower motor neuron (LMN) impairment.

**Methods** We retrospectively collected imaging and clinical data of 47 ALS patients (12 with UMN predominance (UMN-ALS), 16 with LMN predominance (LMN-ALS), and 19 with no clinically defined predominance (Np-ALS)). We further enrolled 23 healthy controls (HC) and 15 ALS mimics (ALS-Mim). These participants underwent brain 3-T magnetic resonance imaging (3-T MRI) with T1-weighted and gradient-echo multi-echo sequences. Automatic segmentation and quantitative susceptibility mapping (QSM) were performed. The skewness of the susceptibility values in the precentral cortex (SuscSKEW) was automatically computed, compared among the groups, and correlated to the clinical variables.

**Results** The Kruskal-Wallis test showed significant differences in terms of SuscSKEW among groups ( $\chi^2(3) = 24.2, p < 0.001$ ), and pairwise tests showed that SuscSKEW was higher in UMN-ALS compared to those in LMN-ALS ( $p < 0.001$ ), HC ( $p < 0.001$ ), Np-ALS ( $p = 0.012$ ), and ALS-Mim ( $p < 0.001$ ). SuscSKEW was highly correlated with the Penn UMN score (Spearman's rho 0.612,  $p < 0.001$ ).

**Conclusion** This study demonstrates that the clinical ALS phenotypes based on UMN/LMN sign predominance significantly differ in terms of magnetic susceptibility properties of the precentral cortex. Combined MRI-histopathology investigations are strongly encouraged to confirm whether this evidence is due to iron overload in UMN-ALS, unlike in LMN-ALS.

## Key Points

- *Magnetic susceptibility in the precentral cortex reflects the prevalence of UMN/LMN impairment in the clinical ALS phenotypes.*
- *The degree of UMN/LMN impairment might be well described by the automatically derived measure of SuscSKEW in the precentral cortex.*
- *Increased SuscSKEW in the precentral cortex is more relevant in UMN-ALS patients compared to those in Np-ALS and LMN-ALS patients.*

**Keywords** Amyotrophic lateral sclerosis · Motor cortex · Motor neuron disease · Magnetic resonance imaging

Giorgio Conte and Valeria Elisa Contarino contributed equally to this work.

✉ Silvia Casale  
casale.silvia88@gmail.com

<sup>1</sup> Neuroradiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, Milan, Italy

<sup>2</sup> Department of Neurology-Stroke Unit and Laboratory of Neuroscience, Istituto Auxologico Italiano IRCCS, piazzale Brescia 20, Milan, Italy

<sup>3</sup> Neuroradiology Unit, ASST Santi Paolo e Carlo, San Carlo Borromeo Hospital, Via Pio II 3, Milan, Italy

<sup>4</sup> Department of Pathophysiology and Transplantation, Università degli Studi di Milano, via Festa del Perdono 7, Milan, Italy

## Abbreviations

ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Revised Amyotrophic Lateral Sclerosis Functional Rating Scale
ALS-Mim	ALS mimics and chameleons
C-ALS	“Classic” ALS
GRE	Gradient-echo
HC	Healthy controls
LMN	Lower motor neuron
LMN-ALS	ALS patients with LMN predominance
Np-ALS	ALS patients with no clinically defined predominance
PLS	Primary lateral sclerosis
PMA	Progressive muscular atrophy
QSM	Quantitative susceptibility mapping
SuscSKEW	Skewness of the susceptibility value distribution in the precentral cortex
UMN	Upper motor neuron
UMN-ALS	ALS patients with UMN predominance

## Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of upper motor neurons (UMN) and/or lower motor neurons (LMN) [1]. “Classic” ALS (C-ALS) is characterized by both UMN and LMN impairment, while atypical forms include primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA), characterized by clinical evidence of isolated UMN or LMN signs, respectively. While the latter phenotypes have been considered separate entities from C-ALS for decades, recent neuropathological studies are supporting a unifying diagnosis of ALS as a spectrum of UMN and LMN degeneration [2].

Magnetic resonance imaging (MRI) with susceptibility-weighted imaging (SWI) has been recently proven to detect changes in susceptibility in the motor cortex of patients affected by ALS, which has been proposed to correspond to the pathological iron accumulation in deep layers of the primary motor cortex [3–5]. Quantitative susceptibility mapping (QSM) algorithms have been used to quantify magnetic susceptibility in the motor cortex in ALS, resulting in a possible radiological marker of UMN burden in ALS [3, 6, 7]. This perspective is attractive for the management of those cases in which the signs of UMN dysfunction are difficult to recognize because of concurrent LMN degeneration, particularly in the early stages of ALS [8, 9].

Susceptibility changes depicted by MRI are not constant and could reflect the clinical prevalence of UMN or LMN signs [10].

The aim of the study was to demonstrate that in the ALS clinical spectrum, the susceptibility properties of the

precentral gyrus investigated by QSM differ according to the UMN/LMN sign predominance.

## Methods

### Study participants

We retrospectively enrolled 47 ALS patients who had consecutively undergone a comprehensive evaluation, including neurological history, neurophysiological assessment, and MRI at the IRCCS Istituto Auxologico Italiano in Milan (Italy), between January 2016 and December 2017. Diagnosis of ALS was made according to the revised El Escorial criteria [1].

Neurological examination was performed by two neurologists with more than 15 years of experience in the assessment of neuromuscular disorders. According to our clinical protocol, the patients underwent MRI within 1 day from the clinical assessment. In addition to the MR images, we collected the following clinical data: predominance of UMN or LMN impairment signs, site of onset (spinal or bulbar), disease duration, Penn UMN score, and score at the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) [11]. The exclusion criteria were as follows: (a) concomitant psychiatric or neurological disorders; (b) the presence of MR imaging artifacts; or (c) brain lesions at the MR imaging involving the motor cortex and corticospinal tracts not related to ALS. The neurologists subgrouped the patients according to the predominance of UMN and LMN impairment, blinded to the imaging data: UMN-predominant ALS (UMN-ALS), LMN-predominant ALS (LMN-ALS), and ALS patients with no clinically defined predominance (Np-ALS). Twelve patients were grouped as UMN-ALS, and six of them who had an absence of LMN signs with a disease duration > 4 years were diagnosed with PLS. Sixteen patients were grouped as LMN-ALS, and eight of them who had an absence of UMN signs with a disease duration > 4 years were diagnosed with PMA. Nineteen patients were grouped as Np-ALS.

We enrolled 23 healthy controls (HC)—volunteers and non-blood relatives of the patients—between January 2018 and September 2018. HC enrollment was performed according to the following criteria: (a) age included in the age range of the ALS group; (b) no history of psychiatric or neurological disorders; and (c) no reported substance abuse. The exclusion criteria were (a) image artifacts in the T1-weighted and/or gradient-echo images or (b) brain MRI showing abnormal findings, except for sporadic small gliotic lesions in the white matter. Study participants partially overlapped with the sample of a previous study focused on the investigation of an unbiased biomarker of the UMN impairment by using a fully automatic paradigm [6].

Additionally, we retrospectively collected images of 15 patients defined as mimics and chameleons (ALS-Mim), as defined by Turner and Talbot [12] who had undergone the same MRI protocol as ALS and HC patients between January 2016 and September 2018. The definite diagnoses of ALS-Mim were extracted from clinical records as follows: two hereditary spastic paraparesis, one metabolic myelopathy with vitamin B<sub>12</sub> deficiency, three corticobasal degeneration, two cervical myeloradiculopathy, five parkinsonism, and two frontotemporal dementia.

Flowchart of the subject's enrollment is shown in Fig. 1.

The research protocol was reviewed and approved by the local ethics committee (Area 2 Milano), in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

### Image acquisition and processing

The acquisition protocol and processing pipeline of the MR images are described in Contarino et al [6] and summarized as follows.

All subjects underwent a brain MRI in a 3-T SIGNA General Electric (GE Healthcare Medical Systems) unit at the IRCCS Istituto Auxologico Italiano in Milan (Italy). The protocol included 3D T1-weighted, 3D fluid-attenuated inversion recovery (FLAIR), T2-weighted fast spin-echo

sequences, and a spoiled multi-echo gradient-echo (GRE) pulse sequence with flow compensation. The echoes were averaged and the phase images were high-pass-filtered by the scanner for clinical purposes. The brain MRIs were collected and assessed by two neuroradiologists (with 4 and 30 years of experience) to define motion artifacts in the T1 or GRE sequences and brain lesions, arriving at a consensus. The whole-brain QSM was calculated from the 3D spoiled gradient-echo pulse sequence using STI Suite [13, 14]. The SPM12 MATLAB Toolbox was used to coregister the QSM map to the 3D T1-weighted image using the normalized mutual information between the 3D T1-weighted and the magnitude image. The 3D T1-weighted images were segmented using FreeSurfer [13, 14], and the “precentral gyrus” and “paracentral lobule” regions-of-interest (ROIs) were merged to build up the precentral cortex ROI shown in Fig. 2. Three representative cases of QSM in the precentral cortex ROI are shown in Figure 1 in Supplemental Material.

The processing time was about 8 h each patient on a 32GB RAM workstation (Intel Xeon W-2145 CPU at 3.70 GHz, 3696 MHz, 8 cores, 16 logical processors), and processing of patients was parallelized to perform 16 patients (according to the number of logical processors) simultaneously. Most of the time is needed for the FreeSurfer segmentation which took about 7 h each patient on a RAM 32.0GB workstation. Processing of several patients was parallelized as much as

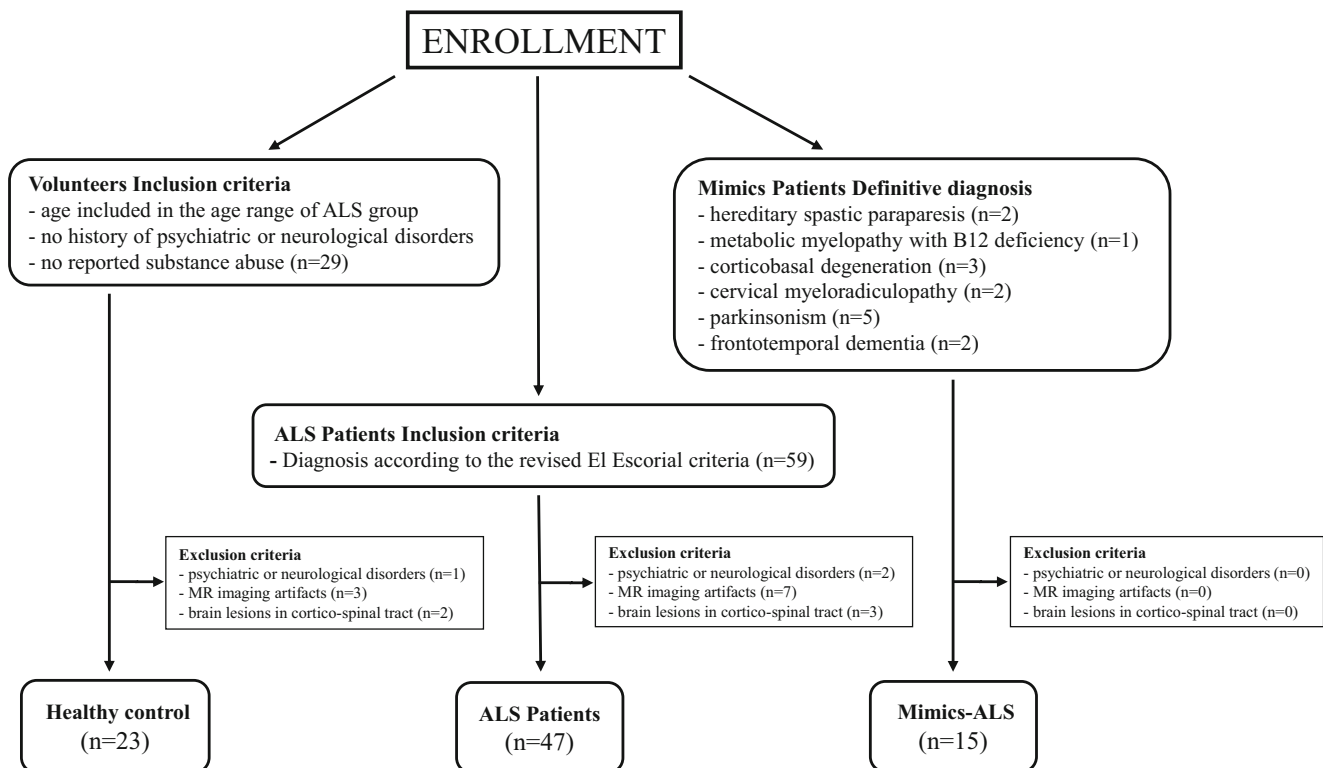
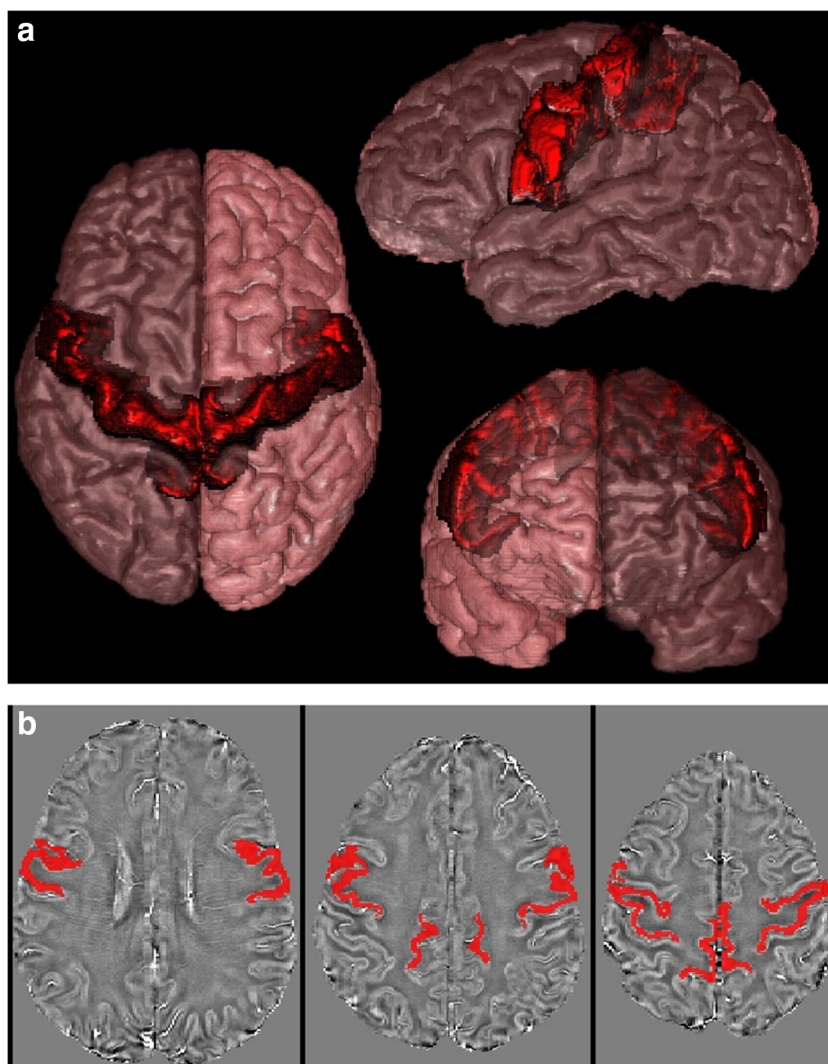


Fig. 1 Flowchart of the subject enrollment

**Fig. 2** **a** 3D reconstruction of the brain. The automatically segmented precentral cortex ROI is highlighted in red. **b** Precentral cortex ROI (red) overlapped on the quantitative susceptibility map. The figure was modified after permission from Contarino et al [6]. ROI, region-of-interest



the number of cores available. The QSM processing takes about a few minutes and the coregistration step about 10–20 min.

The skewness of the susceptibility value distribution in the precentral cortex ROI (SuscSKEW) was calculated using the relative command in MATLAB R2018a (The MathWorks). The susceptibility was chosen among the other statistical metrics according to the previous study [6]. As example, Fig. 3 shows the distributions of the susceptibility values in the precentral cortex in each UMN-ALS (red) and LMN-ALS (blue) patient.

### Statistical analysis

Shapiro-Wilk tests were performed to test the distribution normality of the continuous variables (age, disease duration, ALSFRS-R, and SuscSKEW). The Kruskal-Wallis test was used to compare the continuous variables among the ALS phenotypes, HC, and ALS-Mim, and a chi-square test was

used to compare the categorical variables (disease onset, gender, El Escorial criteria) in the groups. Dunn-Bonferroni tests were carried out for post hoc pairwise comparisons. Rank analysis of covariances (Quade's test) was used to compare SuscSKEW among groups using age as covariate [15].

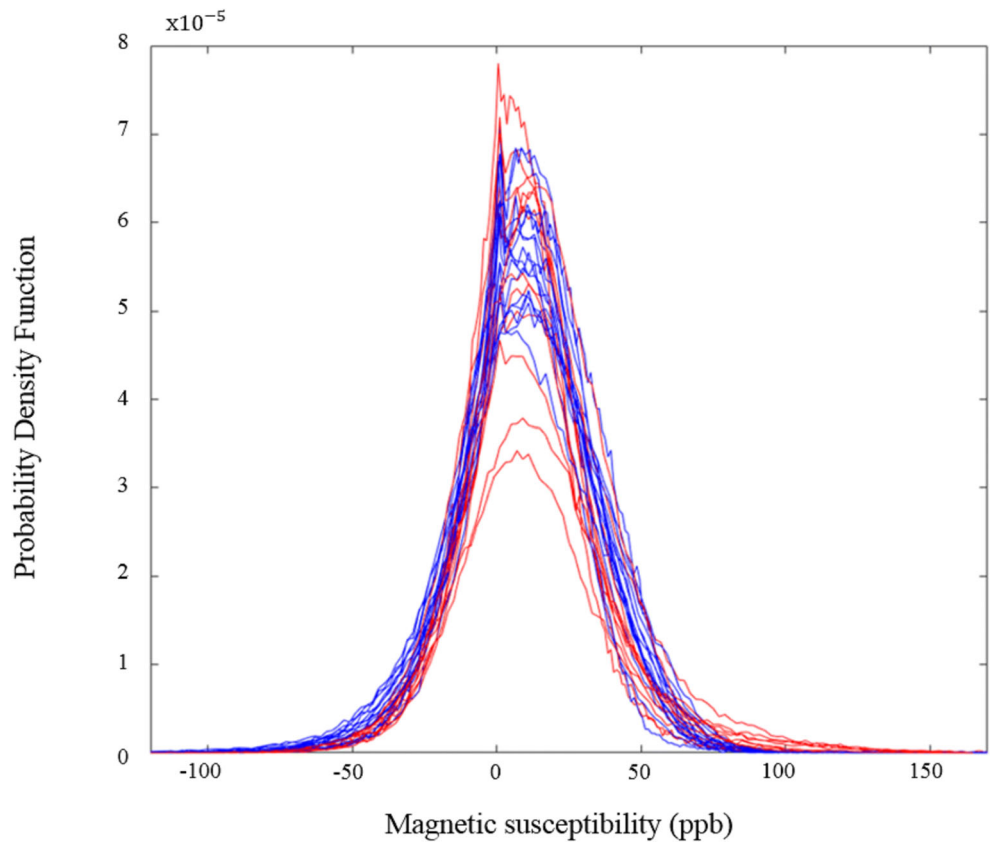
The Spearman test was used to evaluate the correlation between the SuscSKEW and the disease duration, ALSFRS-R, and the UMN score in the ALS patients.

A statistical package for social science (IBM SPSS Statistics, v.25.0.0.1: IBM Corp.) was used to analyze the data. *p* values less than 0.05 were considered significant.

### Results

Nineteen Np-ALS, 12 UMN-ALS, 16 LMN-ALS, 23 HCs, and 15 ALS-Mim were included in the analysis. The groups did not statistically differ in terms of sex ( $\chi^2(4) = 1.24, p = 0.872$ ). The Kruskal-Wallis test showed

**Fig. 3** Susceptibility values in the precentral cortex. Each single-subject susceptibility value distribution is shown as a single curve and colored in red for UMN-ALS patients and blue for LMN-ALS patients. Susceptibility values (ppb) are represented in *x*-axis and probability density function in *y*-axis. LMN-ALS, amyotrophic lateral sclerosis with prevalence of lower motor neuron impairment; UMN-ALS, amyotrophic lateral sclerosis with prevalence of upper motor neuron impairment



a statistically significant difference in terms of age among groups ( $\chi^2(4) = 10.6, p = 0.03$ ), but the pairwise test did not show any statistically significant difference. Np-ALS, UMN-ALS, and LMN-ALS did not statistically differ in terms of El Escorial criteria ( $\chi^2(2) = 5.59, p = 0.252$ ), disease onset ( $\chi^2(2) = 3.39, p = 0.183$ ), ALSFRS-R ( $\chi^2(2) = 0.02, p = 0.990$ ), and disease duration ( $\chi^2(2) = 2.66, p = 0.265$ ). The demographic and clinical data and the SuscSKEW in the subject groups are summarized in Table 1.

The Kruskal-Wallis test showed significant differences in terms of SuscSKEW among groups ( $\chi^2(4) = 26.2 (p < 0.001)$ ), and, in particular, the pairwise tests showed that SuscSKEW was higher in UMN-ALS compared to those in LMN-ALS ( $p < 0.001$ ), HC ( $p < 0.001$ ), Np-ALS ( $p = 0.012$ ), and ALS-Mim ( $p < 0.001$ ). The rank analysis of covariance confirmed significant differences in terms of SuscSKEW among groups after correction for age ( $F(4) = 9.3; p < 0.001$ ).

The UMN score showed a significant correlation with the SuscSKEW (Spearman's rho 0.612,  $p < 0.001$ ). There was no

**Table 1** Demographic data, clinical data, and SuscSKEW in the subject groups. Categorical variables are reported in frequencies, and continuous variables in median and IQR

Demographic, clinical, and imaging variables	Np-ALS <i>n</i> = 19	UMN-ALS <i>n</i> = 12	LMN-ALS <i>n</i> = 16	ALS-Mim <i>n</i> = 15	HC <i>n</i> = 23	<i>p</i> value
Sex (female, male)	12/7	7/5	8/8	8/7	15/8	0.796
Age of patients (years)	58 (50–65)	59 (53–69)	67 (58–72)	70 (54–76)	57 (53–64)	0.06
Disease duration (months)	16 (7–24)	20.5 (18–33)	17 (12–32)			0.25
SuscSKEW	-0.7 (-0.3 to 0.3)	0.3 (0.1–0.6)	-0.2 (-0.3 to 0)	-0.1 (-0.2 to 0)	-0.1 (-0.2 to 0)	<i>0.001</i>

Variable with significant *p* values ( $p < 0.05$ ) is shown in italic

*IQR*, interquartile range; *SuscSKEW*, skewness of the susceptibility value distribution in the precentral cortex; *Np-ALS*, amyotrophic lateral sclerosis with no clinically defined predominance; *HC*, healthy control; *IQR*, interquartile range; *LMN-ALS*, amyotrophic lateral sclerosis with the prevalence of lower motor neuron impairment; *ALS-Mim*, mimics and chameleons; *UMN-ALS*, amyotrophic lateral sclerosis with the prevalence of upper motor neuron impairment

significant correlation between ALSFRS-R score and SuscSKEW (Spearman's  $\rho$  0.016,  $p = 0.928$ ) or between disease duration and SuscSKEW (Spearman's  $\rho$  0.005,  $p = 0.974$ ).

The boxplots of SuscSKEW in the clinical ALS phenotypes based on the UMN/LMN predominance (Np-ALS, UMN-ALS, LMN-ALS) and in the common clinical diagnostic groups, such as C-ALS, PLS, and PMA, grouped by UMN/LMN predominance, are shown in Figs. 4 and 5, respectively.

## Discussion

Our study demonstrated that the magnetic susceptibility in the precentral cortex reflects the prevalence of UMN or LMN in the clinical ALS phenotypes. According to our results, increased susceptibility in the precentral cortex is more relevant in UMN-ALS patients compared to those in Np-ALS and LMN-ALS patients. Indeed, an increase in the skewness means that the right tail of a distribution (in our case, corresponding to higher susceptibility values) is larger than the left tail (lower susceptibility values). As shown in Fig. 2, the right tails of the distributions of the susceptibility values in UMN-ALS patients are enlarged compared to those in the LMN-ALS patients. The higher SuscSKEW of UMN-ALS patients probably reflect an increase in the susceptibility values in

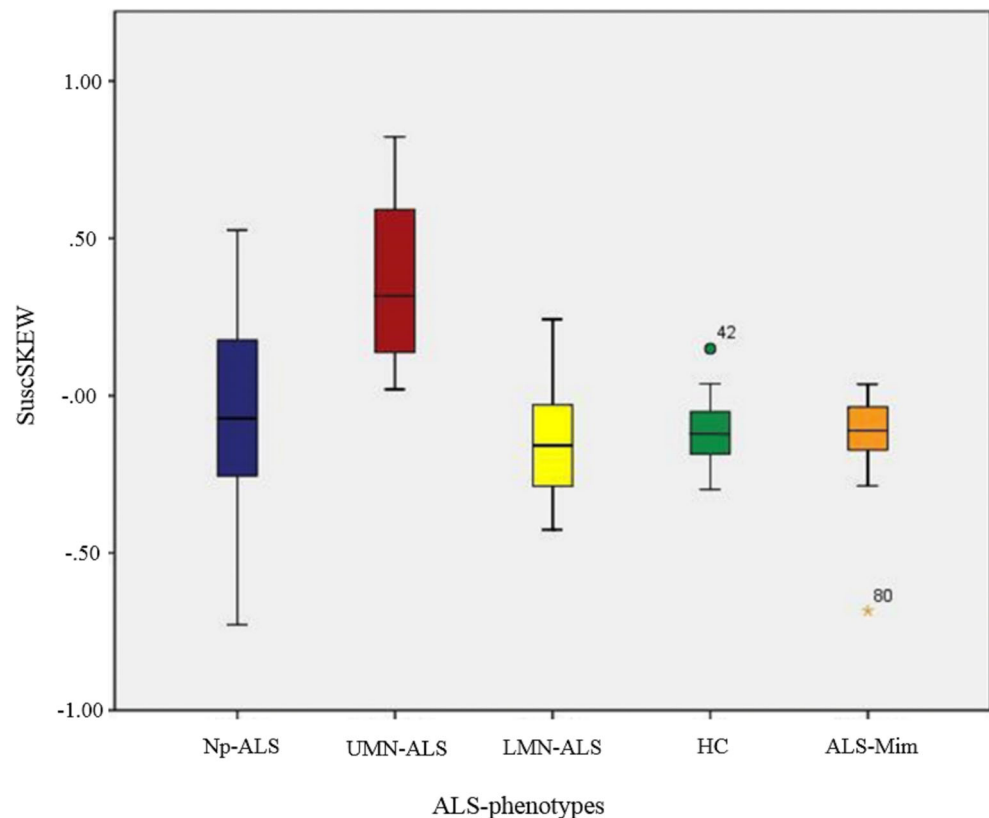
some (even a minority of) voxels within the precentral cortex that are represented in the right tail of the red curves.

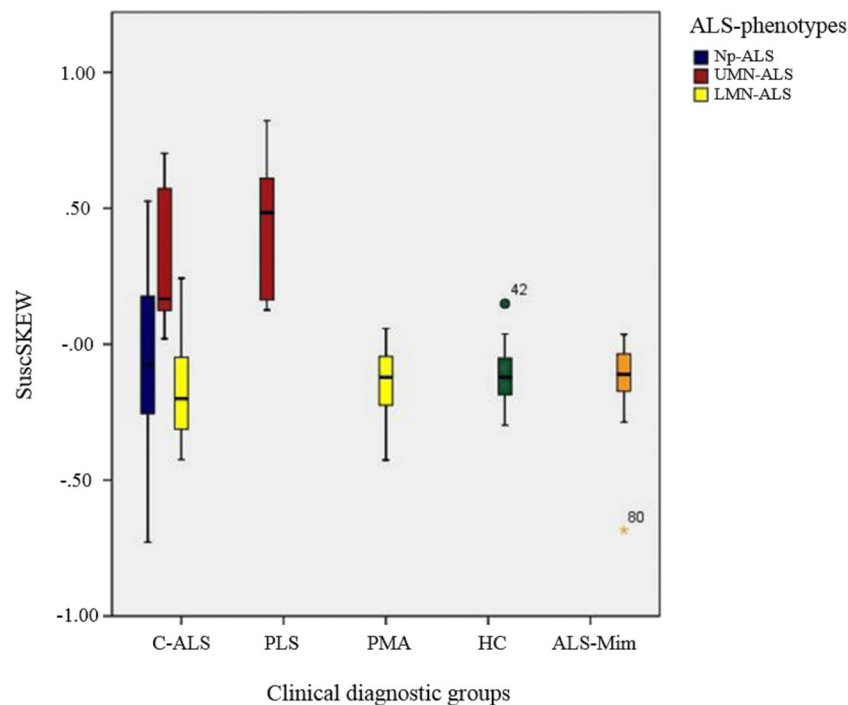
PLS patients showed overlapping SuscSKEW values compared to the remaining patients in the UMN-ALS group, and PMA patients showed overlapping SuscSKEW values compared to the remaining patients in the LMN-ALS group. The clinical predominance of UMN/LMN impairment is consistent with the correlation between UMN scores and SuscSKEW. This evidence supports the classification of PLS and PMA not as distinct nosological entities from ALS, but rather extreme clinical subtypes of ALS [1, 16]. LMN-ALS patients showed SuscSKEW values similar to HCs.

The Np-ALS group shows a wide range of SuscSKEW values, overlapping with those of both the UMN-ALS and LMN-ALS groups. This finding suggests that the Np-ALS group is very heterogeneous in terms of susceptibility in the precentral cortex. The UMN involvement is sometimes difficult to determine clinically, since it can be hidden by signs/symptoms of LMN disease, which explains the discrepancy between the prevalence of UMN involvement at pathology and clinical examination [9].

Considering our observations, we can speculate that the difference in motor cortex susceptibility between UMN-ALS and LMN-ALS might be related to different pathological mechanisms occurring in UMN-ALS and LMN-ALS. Much literature supports the evidence that iron is responsible for an

**Fig. 4** Boxplots of the SuscSKEW in the clinical ALS phenotypes classified by UMN/LMN predominance. The three ALS phenotypes are colored: Np-ALS in blue, UMNALS in red, LMN-ALS in yellow. HC and ALS-Mim are also plotted for comparison and colored in green and orange, respectively. ALS-Mim, ALS mimics and chameleons; HC, healthy control; LMN, lower motor neuron; LMN-ALS, amyotrophic lateral sclerosis with prevalence of lower motor neuron impairment; Np-ALS, amyotrophic lateral sclerosis with no clinically defined predominance; SuscSKEW, skewness of the susceptibility value distribution in the precentral cortex; UMN, upper motor neuron; UMN-ALS, amyotrophic lateral sclerosis with prevalence of upper motor neuron impairment





**Fig. 5** Boxplots of the SuscSKEW in the clinical diagnostic groups. The three common clinical diagnostic groups C-ALS, PLS, and PMA are colored and plotted separately according to the UMN/LMN predominance (Np-ALS in blue, UMN-ALS in red, LMN-ALS in yellow). HC and ALS-Mim are also plotted for comparison and colored in green and orange, respectively. ALS-Mim, mimics and chameleons of amyotrophic lateral sclerosis; C-ALS, classic amyotrophic lateral sclerosis; HC,

healthy control; LMN-ALS, amyotrophic lateral sclerosis with prevalence of lower motor neuron impairment; Np-ALS, amyotrophic lateral sclerosis with no clinically defined predominance; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; SuscSKEW, skewness of the susceptibility value distribution in the precentral cortex; UMN-ALS, amyotrophic lateral sclerosis with prevalence of upper motor neuron impairment

increase in the susceptibility values. While in UMN-ALS iron accumulation might play an important role in the neurodegeneration of UMNs, leading to a metal-mediated oxidative stress, microglia activation, and inflammation, in LMN-ALS, the disorder primarily affects the LMNs in the spinal cord, and UMNs might be subsequently involved via a mechanism unrelated to the above primary iron deposition [17–19]. To our knowledge, there is no histological evidence of iron deposition in the motor cortex of patients with PMA, even if other pathological changes are frequently reported on histology examinations. For example, patients with PMA exhibited macrophages in the cortical spinal tracts in 50% of the cases [20] and degeneration of the cortical spinal tract and loss of Betz cells are shown in 65% and 60%, respectively, of the patients with a clinical diagnosis of PMA [21]. Finally, some studies have demonstrated that a hyper-phosphorylated, ubiquitinated, and cleaved form of TDP-43, which is the major disease protein in ALS, is common in the cerebral cortex or the subcortical white matter of patients with clinical PMA [16, 22]. Riku et al have reported pathological signs of UMN and LMN systems in 85% of patients with PMA, while only the remaining 15% lacked any apparent UMN degeneration [23], possibly because of a secondary degeneration of UMN due to

the degeneration of LMN that might not be linked to iron overload in the primary motor cortex.

Some limitations of our study should be acknowledged. First, the number of cases in PLS, PMA, and ALS-Mim is low, preventing us to test these clinical diagnostic groups for statistical differences. A second limitation is the definition of the region of interest. Because of the small width of the region studied, specifically developed high-resolution sequences would be recommended in order to decrease the partial-volume effects. A third limitation is that no postmortem histopathologic measurements of iron concentration in the precentral cortex were performed to confirm that SuscSKEW increase is due to iron deposition in the precentral cortex. However, previous literature demonstrated that the altered MR signal on gradient-echo T2\*-weighted sequence in the motor cortex of ALS patients is consistent with the pathological iron accumulation

The main advantages of the analysis method here proposed are the following: (1) the MRI sequences used are easy to implement into a routine protocol; (2) the fully automatic pipeline allows the independence of the MRI measures from the users.

Future studies should evaluate the diagnostic value of SuscSKEW in the confirmation of the diagnosis in patients with a prevalence of UMN impairment, especially in early cases. It would be interesting to test whether SuscSKEW

correlates with a histopathological measure of iron deposition in the motor cortex. Future studies are also encouraged to consider a larger group of pathologies that mimic the clinical signs of motor neuron impairment. This would allow us to define the specificity of the automatic measure in order to recognize UMN-ALS against the mimicking disorder. Given the new pharmacological trials testing iron chelators in ALS patients, the use of a reliable automatic pipeline for the magnetic susceptibility analysis of the motor cortex could be useful for the selection of those patients who would benefit most from the administration of the drug and for monitoring the results of these treatments. In recent years, animal studies have investigated the possible therapeutic role of iron chelators, as in murine models of familial and sporadic ALS, demonstrating a decrease in pathologic iron accumulation in the central motor pathway. The iron chelation seems to reduce oxidative stress and increases survival in mice [24–26]. More recently, a pilot trial demonstrated that the use of iron chelators is safe in humans and preliminary results suggest its association with a lower progression disease and a decrease in iron concentration in patients who have had their motor pathways measured by MRI [27]. The user-independent quantitative method proposed in the present study might be useful as a tool to quantify iron deposition in large multicenter studies.

In conclusion, the present study suggests that the clinical spectrum of ALS, as defined by the degree of UMN/LMN impairment, might be well described by the automatically derived measure of magnetic susceptibility in the motor cortex SuscSKEW. Combined MRI and histopathology investigations are now strongly encouraged (1) to investigate the role of the proposed quantitative tool in detecting the increase of the magnetic susceptibility as marker of the iron overload in Np-ALS, and (2) to confirm whether iron overload occurs differently in UMN-ALS and LMN-ALS, as suggested by SuscSKEW.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00330-020-07547-5>.

**Funding** The authors state that this work has not received any funding.

## Compliance with ethical standards

**Guarantor** The scientific guarantor of this publication is Dr. Giorgio Conte, Ospedale Maggiore Policlinico, Neuroradiology Unit.

**Conflict of interest** Prof. Vincenzo Silani receives or has received research supports from the Italian Ministry of Health (Grant RF-201302355764), Fondazione Italiana di Ricerca per la SLA-AriSLA (Grants Exomefals and Novals), Fondazione regionale per la Ricerca Biomedica Regione Lombardia (Project nr. 2015-0023), and E-RARE JTC 2018 (Project Repetomics).

All other authors declare no disclosures of possible conflict of interest and/or commercial involvement involving contents of this manuscript.

**Statistics and biometry** One of the authors has significant statistical expertise.

**Informed consent** Written informed consent was obtained from all subjects (patients) in this study.

**Ethical approval** Institutional Review Board approval was obtained.

**Study subjects or cohorts overlap** Some study subjects or cohorts have been previously reported in Contarino VE, Conte G, Morelli C, et al (2020) “Toward a marker of upper motor neuron impairment in amyotrophic lateral sclerosis: a fully automatic investigation of the magnetic susceptibility in the precentral cortex.” *Eur J Radiol* 124:108815. <https://doi.org/10.1016/j.ejrad.2020.108815>

## Methodology

- retrospective
- observational
- performed at one institution

## References

1. Ludolph A, Drory V, Hardiman O et al (2015) A revision of the El Escorial criteria - 2015. *Amyotroph Lateral Scler Frontotemporal Degener* 16:291–292. <https://doi.org/10.3109/21678421.2015.1049183>
2. Al-Chalabi A, Hardiman O, Kiernan MC, Chiò A, Rix-Brooks B, van den Berg LH (2016) Amyotrophic lateral sclerosis: moving towards a new classification system. *Lancet Neurol* 15:1182–1194
3. Costagli M, Donatelli G, Biagi L et al (2016) Magnetic susceptibility in the deep layers of the primary motor cortex in amyotrophic lateral sclerosis. *Neuroimage Clin* 12:965–969. <https://doi.org/10.1016/j.nicl.2016.04.011>
4. Kwan JY, Jeong SY, Van Gelderen P et al (2012) Iron accumulation in deep cortical layers accounts for MRI signal abnormalities in ALS: correlating 7 tesla MRI and pathology. *PLoS One* 7:e35241. <https://doi.org/10.1371/journal.pone.0035241>
5. Adachi Y, Sato N, Saito Y et al (2015) Usefulness of SWI for the detection of iron in the motor cortex in amyotrophic lateral sclerosis. *J Neuroimaging* 25:443–451
6. Contarino VE, Conte G, Morelli C et al (2020) Toward a marker of upper motor neuron impairment in amyotrophic lateral sclerosis: a fully automatic investigation of the magnetic susceptibility in the precentral cortex. *Eur J Radiol* 124:108815. <https://doi.org/10.1016/j.ejrad.2020.108815>
7. Schweitzer AD, Liu T, Gupta A et al (2015) Quantitative susceptibility mapping of the motor cortex in amyotrophic lateral sclerosis and primary lateral sclerosis. *AJR Am J Roentgenol* 204:1086–1092. <https://doi.org/10.2214/AJR.14.13459>
8. de Carvalho M, Dengler R, Eisen A et al (2008) Electrodiagnostic criteria for diagnosis of ALS. *Clin Neurophysiol* 119:497–503. <https://doi.org/10.1016/j.clinph.2007.09.143>
9. Huynh W, Simon NG, Grosskreutz J, Turner MR, Vucic S, Kiernan MC (2016) Assessment of the upper motor neuron in amyotrophic lateral sclerosis. *Clin Neurophysiol* 127:2643–2660. <https://doi.org/10.1016/j.clinph.2016.04.025>
10. Vázquez-Costa JF, Mazón M, Carreres-Polo J et al (2018) Brain signal intensity changes as biomarkers in amyotrophic lateral sclerosis. *Acta Neurol Scand* 137:262–271. <https://doi.org/10.1111/ane.12863>
11. Cedarbaum JM, Stambler N, Malta E et al (1999) The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments



- of respiratory function. *J Neurol Sci* 169:13–21. [https://doi.org/10.1016/S0022-510X\(99\)00210-5](https://doi.org/10.1016/S0022-510X(99)00210-5)
12. Turner MR, Talbot K (2013) Mimics and chameleons in motor neurone disease. *Pract Neurol* 13:153–164. <https://doi.org/10.1136/practneurol-2013-000557>
  13. Liu C, Li W, Tong KA, Yeom KW, Kuzminski S (2015) Susceptibility-weighted imaging and quantitative susceptibility mapping in the brain. *J Magn Reson Imaging* 42:23–41. <https://doi.org/10.1002/jmri.24768>
  14. Fischl B (2012) FreeSurfer. *Neuroimage* 62:774–781. <https://doi.org/10.1016/j.neuroimage.2012.01.021>
  15. Quade D (1967) Rank analysis of covariance. *J Am Stat Assoc* 62: 1187–1200
  16. Geser F, Stein B, Partain M et al (2011) Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. *Acta Neuropathol* 121:509–517. <https://doi.org/10.1007/s00401-011-0797-z>
  17. Carrí MT, Ferri A, Cozzolino M, Calabrese L, Rutilio G (2003) Neurodegeneration in amyotrophic lateral sclerosis: the role of oxidative stress and altered homeostasis of metals. *Brain Res Bull* 61: 365–374. [https://doi.org/10.1016/s0361-9230\(03\)00179-5](https://doi.org/10.1016/s0361-9230(03)00179-5)
  18. Wang Y, Spincemaille P, Liu Z et al (2017) Clinical quantitative susceptibility mapping (QSM): biometal imaging and its emerging roles in patient care. *J Magn Reson Imaging* 46:951–971. <https://doi.org/10.1002/jmri.25693>
  19. Sheykhsari S, Kozielski K, Bill J et al (2018) Redox metals homeostasis in multiple sclerosis and amyotrophic lateral sclerosis: a review. *Cell Death Dis* 9:348. <https://doi.org/10.1038/s41419-018-0379-2>
  20. Ince PG, Evans J, Knopp M et al (2003) Corticospinal tract degeneration in the progressive muscular atrophy variant of ALS. *Neurology* 60:1252–1258. <https://doi.org/10.1212/01.wnl.0000058901.75728.4e>
  21. Brownell B, Oppenheimer DR, Hughes JT (1970) The central nervous system in motor neurone disease. *J Neurol Neurosurg Psychiatry* 33:338–357. <https://doi.org/10.1136/jnnp.33.3.338>
  22. Nishihira Y, Tan C-F, Hoshi Y et al (2009) Sporadic amyotrophic lateral sclerosis of long duration is associated with relatively mild TDP-43 pathology. *Acta Neuropathol* 117:45–53. <https://doi.org/10.1007/s00401-008-0443-6>
  23. Riku Y, Atsuta N, Yoshida M et al (2014) Differential motor neuron involvement in progressive muscular atrophy: a comparative study with amyotrophic lateral sclerosis. *BMJ Open* 4:e005213. <https://doi.org/10.1136/bmjopen-2014-005213>
  24. Spinelli EG, Agosta F, Ferraro PM et al (2016) Brain MR imaging in patients with lower motor neuron-predominant disease. *Radiology* 280:545–556. <https://doi.org/10.1148/radiol.2016151846>
  25. Jeong SY, Rathore KI, Schulz K, Ponka P, Arosio P, David S (2009) Dysregulation of iron homeostasis in the CNS contributes to disease progression in a mouse model of amyotrophic lateral sclerosis. *J Neurosci* 29:610–619
  26. Golko-Perez S, Amit T, Bar-Am, Youdim MBH, Weinreb O (2017) A novel iron chelator-radical scavenger ameliorates motor dysfunction and improves life span and mitochondrial biogenesis in SOD1G93A ALS mice. *Neurotox Res* 31:230–244
  27. Moreau C, Danel V, Devedjian JC et al (2018) Could Conservative Iron Chelation Lead to Neuroprotection in Amyotrophic Lateral Sclerosis? © Caroline Moreau et al 2018; Published by Mary Ann Liebert, Inc. This Open Access article distributed under the terms of the Creative Commons License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. *Antioxid Redox Signal* 29:742–748

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.