REVIEW

Promises and pitfalls of imaging-based biomarkers in motor neuron diseases

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Purpose of review
Although neuroimaging in motor neuron diseases (MNDs) continues to generate important novel academic insights, the translation of novel radiological protocols into viable biomarkers remains challenging.

Recent findings
A multitude of technological advances contribute to the success of academic imaging in MNDs spanning from the availability of high-field platforms, novel imaging techniques, quantitative spinal cord protocols to whole-brain spectroscopy. International collaborations, protocol harmonization efforts, open-source image analysis suites also fuel developments in the field. Despite the success of academic neuroimaging in MND, the challenge of meaningfully interpreting radiological data from single patients, especially soon after symptom manifestation, and accurately classifying them into relevant diagnostic, phenotypic and prognostic, categories remain challenging, and tracking accruing disease burden over the short follow-up intervals typically utilized in pharmacological trials is also difficult.

Summary
Although we acknowledge the academic achievements of large descriptive studies, an unmet priority of neuroimaging in MND is the development of robust diagnostic, prognostic and monitoring applications to meet the practical demands of clinical decision-making and pharmacological trials. A paradigm shift from group-level analyses to individual-level data interpretation, accurate single-subject classification and disease-burden tracking is therefore urgently needed to distil raw spatially coded imaging data into practical biomarkers.

Keywords
amyotrophic lateral sclerosis, biomarkers, machine-learning, motor neuron disease, neuroimaging, radiology

INTRODUCTION

Quantitative neuroimaging methods are routinely used in clinical trials of multiple sclerosis and increasingly implemented in pharmacological trials of neurodegenerative conditions. Despite considerable advances in computational imaging in motor neuron diseases (MNDs), it has primarily contributed academic insights to date on phenotype and genotype-associated signatures, presymptomatic alterations, propagation patterns and so on and has not been developed into viable clinical markers with pragmatic utility in diagnostic, prognostic and clinical trial applications. The gap between the success of academic studies and the lack of translation into pragmatic, routine clinical protocols is striking. One of the examples that epitomises this contradiction is the achievements of large, group-level studies, often with hundreds of participants who succeed in characterizing disease-specific traits [1▪▪,2], and the persistent challenge of meaningfully interpreting single-patient data from specific individuals [3,4]. Developments in MND imaging are best discussed from a dual technological and clinical perspective. The relentless methodological advances in neuroimaging enable unprecedented single-to-noise ratios, spatial resolution, fast acquisition times and so on facilitating richer and higher quality raw data. On the contrary, conceptual developments have helped to shift the focus from

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KEY POINTS

- Computational neuroimaging contributed important academic insights into phenotype and genotype-associated signatures and propagation patterns in motor neuron disease.
- High-field MR platforms, quantitative spinal cord protocols, whole-brain spectroscopy and novel white matter imaging techniques gave new momentum to motor neuron disease imaging.
- International collaborations, protocol harmonization efforts, the availability of robust open-source image analysis suites also fuelled developments in biomarker development.
- The accurate classification of single imaging datasets from individual patients into relevant diagnostic, phenotypic and prognostic categories herald viable clinical applications.
- Disease burden quantification and longitudinal radiological assessments should be incorporated into pharmacological trial designs to monitor disease progression and assess response to therapy.

pursuing descriptive studies to develop models with potential practical utility such as prognostic indicators, single-subject classification and identification of clusters with unique radiological, clinical and genetic characteristics. Accordingly, we review recent developments in MND imaging from a dual academic-clinical perspective with an earnest effort to identify the barriers of translating methodological advances into viable biomarkers.

PHENOTYPIC HETEROGENEITY

Motor neuron disease imaging continues to be dominated by studies of amyotrophic lateral sclerosis, despite the clinical relevance of accurately categorising paucisymptomatic patients into the appropriate diagnostic categories before fulfilling clinical criteria. Primary lateral sclerosis is a notoriously difficult diagnosis to establish given its overlapping clinical features with UMN-predominant ALS [5,6▪,7], but the recently published diagnostic criteria now permit the labelling of early PLS patients as 'probable PLS' [8]. The imaging studies of these 'early' PLS cohorts have revealed a pattern consistent with what is now called 'definite PLS' validating the utility of the new criteria [9,10]. Hereditary spastic paraparesis (HSP) has overlapping clinical features with PLS and the marked corticospinal, corpus callosum and, depending on genotype, cerebellar degeneration is also reminiscent of some of the radiological alterations observed in ALS [11,12,13▪▪,14]. Low-incidence ALS mimics, such as postpolio syndrome, was traditionally associated with widespread cerebral disease, but recent imaging studies have highlighted the lack of atrophy both in supra-tentorial and infra-tentorial regions [15,16]. Kennedy's disease or spinal and bulbar muscular atrophy (SBMA) has also been increasingly evaluated by computation imaging studies and some degree of cerebral involvement has been consistently captured [17]. Other LMN conditions such as adult-onset SMA are also increasingly evaluated radiologically, conditions wherein the implementation of novel spinal protocols may be particularly pertinent [18,19]. Although descriptive studies of specific MND variants along the LMN-UMN spectrum are of undeniable academic interest, the challenge of mathematically classifying single patients into these categories remains notoriously challenging [4,20–23]. The pursuit of robust classification studies seem like an obvious clinical priority given the strikingly divergent survival profile of these MND subtypes, for example PLS vs. ALS [5].

EXTRA-MOTOR ALTERATIONS

Imaging studies of MNDs have traditionally focused on primary motor regions such as the precentral gyrus, brainstem and corticospinal tracts [13▪▪,24–26]. In recent years, considerable effort has been made to the characterization of extra-pyramidal motor involvement, and nigrostriatal, cerebellar, basal ganglia, pre and supplementary motor regions have been increasingly evaluated [12,27,28▪,29,30,31]. These studies have clearly demonstrated that gait impairment, decline in dexterity, dysarthria and dysphagia are not solely driven by pyramidal degeneration, therefore seeking direct correlations between motor cortex or corticospinal tract measures and functional disability scores may be erroneous [32]. Recent imaging studies departed from the evaluation of motor networks altogether and a series of articles have focused on the characterization of frontotemporal, thalamic, parietal and occipital alterations in MND cohorts [33▪,34▪▪,35–37].

METHODOLOGICAL ADVANCES

In addition to specific methodological advances, the progressive separation of research and clinical imaging facilities allows the implementation of dedicated research protocols, which are often much longer. The widespread availability of 3T platforms led to the generation of large, high-quality data sets and the first publications of ultra-high field platforms are now also emerging from 7 Tesla scanners [38–40]. PET data are increasingly acquired in

conjunction with MRI data and contributed important metabolic insights to complement MR-based structural analyses [41,42]. Imaging studies of MND have traditionally relied on a high-resolution 3D structural T1-wieghted and a diffusion-weighted imaging to evaluate white mater alterations. Although these two elements remain at the core of most protocols, a number of novel and innovative imaging methods have now been trialled and have proven remarkably successful in generating additional insights. White matter imaging in MND has moved beyond standard DWI/DTI and NODDI has emerged as a powerful tool with seemingly superior detection sensitivity both in symptomatic and presymptomatic MND cohorts [43–45]. High spatial-resolution DTI and non-Bayesian whiter diffusion sequences are increasingly trialled in MND, as they more offer a more nuanced characterization of crossing fibres [44]. Muscle imaging is another intriguing frontier of MND imaging, which, while not performed routinely, provides a measure of muscle degeneration secondary to denervation and such as indirect proxy of LMN involvement [46,47]. Spinal cord MRI is one of the success stories of MND imaging [48,49]; it has captured presymptomatic changes in *C9orf72* mutation carriers [50], metabolic alterations in *SOD1* carriers [51], shown associations with respiratory compromise [52], correlations with clinical disability [48,53,54] and captures both the UMN and anterior horn aspects of ALS [55–59]. Spinal DTI protocols captured both descending corticospinal and ascending sensory tract degeneration in ALS [53,56,57,60–63]. Spinal magnetization transfer imaging (MTI) also detected accruing cord disease in ALS [53,56,57]. Cord spectroscopy yielded revealed considerable clinico-radiological associations [51,64,65]. Methodological advances in spinal imaging, meticulous respiratory and cardiac gating and improved analysis suites, led to improved noise filtering, spatial registration and raw data quality, which in turn led to clinically relevant research findings [48]. As with other emerging imaging modalities, quantitative spinal imaging is yet to filter down to everyday clinical and pharmacological applications. Quantitative susceptibility mapping or QSM is other modality that has been increasingly explored by academic imaging studies and has added interesting academic insights re cerebral iron deposition. In specific applications, such as studies centred on ferroptosis or iron-chelation, these modalities may be of particular interest [66]. Sodium imaging is yet another innovative approach, which has only been recently applied to ALS cohorts and seems to have excellent detection sensitivity for neurodegenerative change [67]. MRS spectroscopy has long been applied to ALS cohorts, but voxel placement was traditionally centred on motor areas in single-voxel protocols [68▪▪]. With the recognition of frontotemporal change in ALS and PLS, single voxel techniques have been increasingly applied outside the motor cortex [34▪▪]. With the advent of robust whole-brain multivoxel protocols, metabolic alterations can now be assessed over the entire cerebrum [69,70]. As with other modalities, fMRI was initially used in studies with motor paradigm, before trialled in cognitive and sensory paradigm, and more recently, resting fMRI is routinely recorded, which permits posthoc connectivity analyses [24,71].

ADVANCES IN STUDY DESIGN

In addition to methodological advances, significant advances took place in study design. The traditional approach of characterizing large symptomatic cohorts stratified by genotype or phenotype is increasingly superseded by smaller studies of homogenous clinical or demographic characteristics. The caveat of recruiting unselected patients into a single study group is that they are likely to be in different stages of their disease, have different phenotypic traits, different clinical characteristics both in terms of motor disability and cognitive profile. The resulting statistical maps of such studies are unlikely to be representative of specific disease subgroups. At the very least, in single-group descriptive studies, patients should ideally be relatively uniform in their symptom duration or disability profile. More recent studies have departed from large cross-sectional study designs and increasingly rely on multitime-point longitudinal analyses, which are not only ideal to map progressive changes, but they also enable the ranking of various radiological measures in their sensitivity to capture progressive changes in relatively short follow-up intervals [22,72]. Two-time point longitudinal studies are ill-suited to characterize longitudinal trajectories and progressive changes may be misconstrued as linear decline, which is unlikely to represent the dynamics of underlying pathological processes. Multitime-point studies, on the contrary, may detect curvilinear changes and confirm the ceiling or flooring- effects of specific integrity measures. There is a notion that white matter degeneration – or at least its radiological proxies – may be a relatively early feature of ALS, as corticospinal and corpus callosum changes can be readily captured around the time of the diagnosis [73]. These attributes make white matter measures good diagnostic markers; however, several studies indicate that they may exhibit limited further change on follow-up making them relatively ill-suited for monitoring purposes. Grey matter

measures, on the contrary, can be relatively preserved around the time of diagnosis, but often display progressive change over the clinical course of ALS. These observations are further supported by presymptomatic studies wherein white matter changes and subcortical grey matter changes are often detected before cortical involvement [45,74]. It is therefore increasingly clear that no single imaging index can address the biomarker requirements of both diagnostic and monitoring applications and that a panel of several markers with complementary detection profiles (early vs. longitudinal) are required. One of the most exciting facets of imaging-based studies in MND is the increasing availability of presymptomatic data sets [75]. Early proof-of-concept presymptomatic studies often relied on admixed *SOD1-C9orf72* cohorts to demonstrate ALS-associated changes before symptom onset [76–79], but these studies have now been superseded by large genotype-specific studies. Corticospinal tract degeneration [80], superior spinal cord NAA reductions [51] and fronto-temporal hypometabolism [81] were described in asymptomatic *SOD1* carriers. Frontotemporal, parietal, cerebellar and subcortical grey matter changers [82–85] have been described in *C9orf72* hexanucleotide repeat expansion carriers. Corticospinal [50,85], commissural, orbitofrontal, cingulate and uncinate degeneration is also a common white matter finding [45,74,85,86]. Neurite orientation dispersion and density imaging (NODDI) captured early white matter degeneration [45] and presymptomatic frontotemporal and subcortical hypometabolism has also been described [41]. To account for motor disability, motor imagery is increasingly used instead of motor paradigms and has been successful in capturing network level connectivity alterations in motor circuits [27,28▪].

FROM GROUP-LEVEL ANALYSES TO SINGLE PATIENT DATA INTERPRETATION

In recognition of the importance to interpreting single data sets from individual patients, a multitude of statistical approaches has been trialled in MND. Relatively simple strategies, such as *z*-scoring with reference to demographically matched controls [22,87], or discriminant function analyses [88], were soon superseded by more complex machine-learning models [20]. Machine-learning algorithms often rely on a selection of best discrimination MR features to reduce computational needs and simplify models without sacrificing classification accuracy [88–90]. Imaging-based machine learning models in MND often include a variety of structural, diffusivity and functional indices. Machine learning strategies are often discussed in terms of 'supervised' and 'unsupervised' approaches. Unsupervised models, such as clustering (K-means, hierarchical, probabilistic) or dimensionality reduction strategies (principal component analysis, singular value decomposition) may uncover naturally occurring data patterns without *a priori* hypotheses and reliance on carefully labelled training data. Supervised learning algorithms such as linear regression, logistic regression, naive bayes, support vector machines (SVMs), decision trees, K-nearest neighbour algorithms, random forests and so on need well labelled training data for accurate classification [21,90]. Although binary classification into 'healthy' vs. 'ALS' categories was relatively successful [88], the accurate categorization of patients with various MND phenotypes has proven more challenging [4]. Predicting prognosis in individual patients based on single-subject clinical or imaging data is another exciting frontier of ALS research with huge practical relevance and few promising studies have already been published [3,21,59,91,92▪].

SCRUTINIZING ACADEMIC CONCEPTS

With ever-improving spatial resolution, the implementation of network-level analyses and the unprecedented detection sensitivity of novel metabolic, diffusivity and connectomics methods, imaging is now well placed to examine prevailing biological concepts such as stage-wise propagation, corticofugal spread, trans-synaptic propagation, network-wise degeneration, neuroplasticity, motor reserve, cognitive reserve and disease clusters [93–95,96▪,97]. Several of these concepts were originally based on observations from animal studies or extrapolated from postmortem data; therefore, one of the roles of advanced imaging in ALS is the critical appraisal of these concepts *in vivo* in human studies. Although disease-burden patterns have been repeatedly mapped to pathological stages [98,99], large multimodal longitudinal studies are still required, ideally spanning from the presymptomatic phase to phenoconversion, to dissect the trans-synaptic, metabolic, inflammatory and developmental components of disease propagation.

RESEARCH PRIORITIES

Although the continued publication of ever larger and methodologically complex descriptive studies is exciting, ultimately, the emergence of actionable single-subject data interpretation frameworks will bring the long-awaited transition from academic studies to viable applications with genuine clinical utility. Any framework that reliable detects MND-associated changes and classifies an individual

subject into clinically relevant phenotypic, survival or prognostic categories while distinguishing him from common clinical mimics would offer practical benefits and potentially curtail the notoriously long diagnostic journey most patients with MND face [21,91]. Similarly, any combination of imaging-derived metrics that allow the precision tracking of accruing disease-burden would be a welcome addition to the monitoring panels of clinical trials. Single-centre machine-learning initiatives have provided promising proof-of-concept data, but these have to be validated by large blinded, extraneous data sets, potentially acquired on different scanning platforms. The superiority of radiological markers has to be demonstrated against cheaper serum or CSF-derived wet biomarkers or bed-side clinical measures or rating scales. The practical drawbacks of imaging in MND, such as accessibility, high attrition rates, poor tolerability, expenses, the complexity of interpretation have to be addressed with candour, and the results of negative studies need to be disseminated as a lesson for future studies.

CONCLUSION

Neuroimaging in MND has benefited from both momentous methodological advances and the long-awaited departure from phenotypically admixed descriptive studies. Given the plethora of confirmatory cross-sectional studies, a clear priority of MND imaging is the accurate classification of single-subject data from individual patients, early phenotypical categorization, prognostic modelling and phenoconversion prediction in asymptomatic mutation carriers. Longitudinal studies of large cohorts have to be superseded by studies that can accurately map disease propagation in individual subjects, so that these methods may be implemented in clinical trials. The pursuit of direct correlations between cerebral measures and motor disability seems naive, as LMN involvement contributes significantly and sometime disproportionately to clinical disability. Although MR spectroscopy seems to be underutilized, findings from fMRI findings may be over-interpreted. Studies should ideally incorporate a multimodal panel of structural, diffusion, functional and metabolic metrics and a combination of cerebral, spinal and muscle protocols should be implemented to cover the entire neuro-axis from cortex to muscle.

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Conflicts of interest

The authors have no conflicts of interest to declare.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Tan HHG, Westeneng HJ, Nitert AD, *et al.* MRI clustering reveals three ALS ■■ subtypes with unique neurodegeneration patterns. Ann Neurol 2022; 92:1030–1045.
Cluster analysis of a large MR data sets reveals unique subgroups.
2. van der Burgh HK, Westeneng HJ, Meier JM, *et al.* Cross-sectional and longitudinal assessment of the upper cervical spinal cord in motor neuron disease. Neuroimage Clin 2019; 24:101984.
3. van der Burgh HK, Schmidt R, Westeneng HJ, *et al.* Deep learning predictions of survival based on MRI in amyotrophic lateral sclerosis. Neuroimage Clin 2017; 13:361–369.
4. Bede P, Murad A, Lope J, *et al.* Phenotypic categorisation of individual subjects with motor neuron disease based on radiological disease burden patterns: a machine-learning approach. J Neurol Sci 2021; 432:120079.
5. Finegan E, Chipika RH, Shing SLH, *et al.* Primary lateral sclerosis: a distinct entity or part of the ALS spectrum? Amyotroph Lateral Scler Frontotemporal Degener 2019; 20:133–145.
6. Finegan E, Siah WF, Li Hi Shing S, *et al.* Cerebellar degeneration in primary ■ lateral sclerosis: an under-recognized facet of PLS. Amyotroph Lateral Scler Frontotemporal Degener 2022; 23:542–553.
Primary lateral sclerosis involves the cerebellum and not just the primary motor regions.
7. Bede P, Pradat PF, Lope J, *et al.* Primary lateral sclerosis: clinical, radiological and molecular features. Rev Neurol (Paris) 2021; 178:196–205.
8. Turner MR, Barohn RJ, Corcia P, *et al.* Primary lateral sclerosis: consensus diagnostic criteria. J Neurol Neurosurg Psychiatry 2020; 91:373–377.
9. Finegan E, Li Hi Shing S, Siah WF, *et al.* Evolving diagnostic criteria in primary lateral sclerosis: the clinical and radiological basis of 'probable PLS'. J Neurol Sci 2020; 417:117052.
10. Finegan E, Siah WF, Shing SLH, *et al.* Imaging and clinical data indicate considerable disease burden in 'probable' PLS: patients with UMN symptoms for 2-4 years. Data in brief 2020; 106247.
11. Mulkerrin G, França MC Jr, Lope J, *et al.* Neuroimaging in hereditary spastic paraplegias: from qualitative cues to precision biomarkers. Expert Rev Mol Diagn 2022; 22:745–760.
12. Bede P, Chipika RH, Christidi F, *et al.* Genotype-associated cerebellar profiles in ALS: focal cerebellar pathology and cerebro-cerebellar connectivity alterations. J Neurol Neurosurg Psychiatry 2021; 92:1197–1205.
13. Tahedl M, Tan EL, Chipika RH, *et al.* Brainstem-cortex disconnection in ■■ amyotrophic lateral sclerosis: bulbar impairment, genotype associations, asymptomatic changes and biomarker opportunities. J Neurol 2023. AQ6
Connectivity metrics capture progressive neurodegeneration in ALS.
14. Pioro EP, Turner MR, Bede P. Neuroimaging in primary lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2020; 21:18–27.
15. Li Hi Shing S, Lope J, McKenna MC, *et al.* Increased cerebral integrity metrics in poliomyelitis survivors: putative adaptation to longstanding lower motor neuron degeneration. J Neurol Sci 2021; 424:117361.
16. Shing SLH, Murad A, Lope J, *et al.* Cerebellar remodelling decades after spinal cord insult: neuroplasticity in poliomyelitis survivors. J Integr Neurosci 2021; 21:65.
17. Pradat PF, Bernard E, Corcia P, *et al.* The French national protocol for Kennedy's disease (SBMA): consensus diagnostic and management recommendations. Orphanet J Rare Dis 2020; 15:90.
18. Querin G, El Mendili MM, Lenglet T, *et al.* The spinal and cerebral profile of adult spinal-muscular atrophy: a multimodal imaging study. Neuroimage Clin 2019; 21:101618.

19. Lebouteux MV, Franques J, Guillevin R, *et al.* Revisiting the spectrum of lower motor neuron diseases with snake eyes appearance on magnetic resonance imaging. Eur J Neurol 2014; 21:1233–1241.
20. Grollemund V, Pradat PF, Querin G, *et al.* Machine learning in amyotrophic lateral sclerosis: achievements, pitfalls, and future directions. Front Neurosci 2019; 13:135.
21. Schuster C, Hardiman O, Bede P. Survival prediction in amyotrophic lateral sclerosis based on MRI measures and clinical characteristics. BMC Neurol 2017; 17:73.
22. Tahedl M, Li Hi Shing S, Finegan E, *et al.* Propagation patterns in motor neuron diseases: individual and phenotype-associated disease-burden trajectories across the UMN-LMN spectrum of MNDs. Neurobiol Aging 2021; 109:78–87.
23. Tahedl M, Li Hi Shing S, Finegan E, *et al.* Imaging data reveal divergent longitudinal trajectories in PLS, ALS and poliomyelitis survivors: group-level and single-subject traits. Data in brief 2021; 39:107484.
24. Tahedl M, Tan EL, Shing SLH, *et al.* Not a benign motor neuron disease: longitudinal imaging captures relentless motor connectome disintegration in primary lateral sclerosis. Eur J Neurol 2023; 30:1232–1245.
25. Chipika RH, Finegan E, Li Hi Shing S, *et al.* Tracking a fast-moving disease: longitudinal markers, monitoring, and clinical trial endpoints in ALS. Front Neurol 2019; 10:229.
26. Bede P, Chipika RH, Finegan E, *et al.* Brainstem pathology in amyotrophic lateral sclerosis and primary lateral sclerosis: a longitudinal neuroimaging study. Neuroimage Clin 2019; 24:102054.
27. Abidi M, de Marco G, Grami F, *et al.* Neural correlates of motor imagery of gait in amyotrophic lateral sclerosis. J Magn Reson Imaging 2021; 53:223–233.
28. ■ Abidi M, Pradat PF, Termoz N, *et al.* Motor imagery in amyotrophic lateral sclerosis: an fMRI study of postural control. Neuroimage Clin 2022; 35:103051.
Motor imagery enables the functional assessment of motor networks in patients with considerable disability.
29. Feron M, Couillandre A, Mseddi E, *et al.* Extrapyramidal deficits in ALS: a combined biomechanical and neuroimaging study. J Neurol 2018; 265:2125–2136.
30. McKenna MC, Corcia P, Couratier P, *et al.* Frontotemporal pathology in motor neuron disease phenotypes: insights from neuroimaging. Front Neurol 2021; 12:723450.
31. Bede P, Omer T, Finegan E, *et al.* Connectivity-based characterisation of subcortical grey matter pathology in frontotemporal dementia and ALS: a multimodal neuroimaging study. Brain Imaging Behav 2018; 12:1696–1707.
32. Verstraete E, Turner MR, Grosskreutz J, *et al.* Mind the gap: the mismatch between clinical and imaging metrics in ALS. Amyotroph Lateral Scler Frontotemporal Degener 2015; 16:524–529.
33. ■ Chipika RH, Mulkerrin G, Murad A, *et al.* Alterations in somatosensory, visual and auditory pathways in amyotrophic lateral sclerosis: an under-recognised facet of ALS. J Integr Neurosci 2022; 21:88.
Sensory pathways and networks are not spared in ALS.
34. ■■ Christidi F, Argyropoulos GD, Karavasilis E, *et al.* Hippocampal metabolic alterations in amyotrophic lateral sclerosis: a magnetic resonance spectroscopy study. Life (Basel, Switzerland) 2023; 13:571.
MRS can be meaningfully applied to frontotemporal brain regions in ALS.
35. Chipika RH, Christidi F, Finegan E, *et al.* Amygdala pathology in amyotrophic lateral sclerosis and primary lateral sclerosis. J Neurol Sci 2020; 417:117039.
36. Chipika RH, Finegan E, Li Hi Shing S, *et al.* Switchboard' malfunction in motor neuron diseases: selective pathology of thalamic nuclei in amyotrophic lateral sclerosis and primary lateral sclerosis. Neuroimage Clin 2020; 27:102300.
37. Christidi F, Karavasilis E, Rentzos M, *et al.* Hippocampal pathology in amyotrophic lateral sclerosis: selective vulnerability of subfields and their associated projections. Neurobiol Aging 2019; 84:178–188.
38. Verstraete E, Biessels GJ, van Den Heuvel MP, *et al.* No evidence of microbleeds in ALS patients at 7 Tesla MRI. Amyotroph Lateral Scler 2010; 11:555–557.
39. Atassi N, Xu M, Triantafyllou C, *et al.* Ultra high-field (7tesla) magnetic resonance spectroscopy in amyotrophic lateral sclerosis. PLoS One 2017; 12:e0177680.
40. Barry RL, Babu S, Anteraper SA, *et al.* Ultra-high field (7T) functional magnetic resonance imaging in amyotrophic lateral sclerosis: a pilot study. Neuroimage Clin 2021; 30:102648.
AQ7 41. De Vocht J, Blommaert J, Devrome M, *et al.* Use of multimodal imaging and clinical biomarkers in presymptomatic carriers of C9orf72 repeat expansion. JAMA Neurol 2020; 77:1008–1017.
42. Canosa A, Vacchiano V, D'Ovidio F, *et al.* Brain metabolic correlates of apathy in amyotrophic lateral sclerosis: an 18F-FDG-positron emission tomography stud. Eur J Neurol 2021; 28:745–753.
43. Broad RJ, Gabel MC, Dowell NG, *et al.* Neurite orientation and dispersion density imaging (NODDI) detects cortical and corticospinal tract degeneration in ALS. J Neurol Neurosurg Psychiatry 2019; 90:404–411.
44. Barritt AW, Gabel MC, Cercignani M, Leigh PN. Emerging magnetic resonance imaging techniques and analysis methods in amyotrophic lateral sclerosis. Front Neurol 2018; 9:1065.
45. Wen J, Zhang H, Alexander DC, *et al.* Neurite density is reduced in the presymptomatic phase of C9orf72 disease. J Neurol Neurosurg Psychiatry 2019; 90:387–394.
46. Jenkins TM, Alix JJP, David C, *et al.* Imaging muscle as a potential biomarker of denervation in motor neuron disease. J Neurol Neurosurg Psychiatry 2017; 89:248–255.
47. Jenkins TM, Burness C, Connolly DJ, *et al.* A prospective pilot study measuring muscle volumetric change in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14:414–423.
48. El Mendili MM, Querin G, Bede P, Pradat PF. Spinal cord imaging in amyotrophic lateral sclerosis: historical concepts-novel techniques. Front Neurol 2019; 10:350.
49. Bede P, Bokde AL, Byrne S, *et al.* Spinal cord markers in ALS: diagnostic and biomarker considerations. Amyotroph Lateral Scler 2012; 13:407–415.
50. Querin G, Bede P, El Mendili MM, *et al.* Presymptomatic spinal cord pathology in c9orf72 mutation carriers: a longitudinal neuroimaging study. Ann Neurol 2019; 86:158–167.
51. Carew JD, Nair G, Andersen PM, *et al.* Presymptomatic spinal cord neurometabolic findings in SOD1-positive people at risk for familial ALS. Neurology 2011; 77:1370–1375.
52. Grolez G, Kyheng M, Lopes R, *et al.* MRI of the cervical spinal cord predicts respiratory dysfunction in ALS. Sci Rep 2018; 8:1828.
53. Cohen-Adad J, El Mendili MM, Morizot-Koutlidis R, *et al.* Involvement of spinal sensory pathway in ALS and specificity of cord atrophy to lower motor neuron degeneration. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14:30–38.
54. Iglesias C, Sangari S, El Mendili MM, *et al.* Electrophysiological and spinal imaging evidences for sensory dysfunction in amyotrophic lateral sclerosis. BMJ Open 2015; 5:e007659.
55. Branco LM, De Albuquerque M, De Andrade HM, *et al.* Spinal cord atrophy correlates with disease duration and severity in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2014; 15:93–97.
56. El Mendili MM, Cohen-Adad J, Pelegrini-Issac M, *et al.* Multiparametric spinal cord MRI as potential progression marker in amyotrophic lateral sclerosis. PLoS One 2014; 9:e95516.
57. Rasoanandrianina H, Grapperon AM, Taso M, *et al.* Region-specific impairment of the cervical spinal cord (SC) in amyotrophic lateral sclerosis: a preliminary study using SC templates and quantitative MRI (diffusion tensor imaging/inhomogeneous magnetization transfer). NMR Biomed 2017; 30:.
58. Paquin ME, El Mendili MM, Gros C, *et al.* Spinal cord gray matter atrophy in amyotrophic lateral sclerosis. AJNR Am J Neuroradiol 2018; 39:184–192.
59. Querin G, El Mendili MM, Lenglet T, *et al.* Spinal cord multiparametric magnetic resonance imaging for survival prediction in amyotrophic lateral sclerosis. Eur J Neurol 2017; 24:1040–1046.
60. Nair G, Carew JD, Usher S, *et al.* Diffusion tensor imaging reveals regional differences in the cervical spinal cord in amyotrophic lateral sclerosis. NeuroImage 2010; 53:576–583.
61. Agosta F, Rocca MA, Valsasina P, *et al.* A longitudinal diffusion tensor MRI study of the cervical cord and brain in amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2009; 80:53–55.
62. Valsasina P, Agosta F, Benedetti B, *et al.* Diffusion anisotropy of the cervical cord is strictly associated with disability in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2007; 78:480–484.
63. Wang Y, Liu L, Ma L, *et al.* Preliminary study on cervical spinal cord in patients with amyotrophic lateral sclerosis using MR diffusion tensor imaging. Acad Radiol 2014; 21:590–596.
64. Ikeda K, Murata K, Kawase Y, *et al.* Relationship between cervical cord 1H-magnetic resonance spectroscopy and clinoco-electromyographic profile in amyotrophic lateral sclerosis. Muscle Nerve 2013; 47:61–67.
65. Carew JD, Nair G, Pineda-Alonso N, *et al.* Magnetic resonance spectroscopy of the cervical cord in amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2011; 12:185–191.
66. Devos D, Moreau C, Kyheng M, *et al.* A ferroptosis-based panel of prognostic biomarkers for amyotrophic lateral sclerosis. Sci Rep 2019; 9:2918.
67. Grapperon AM, Ridley B, Verschueren A, *et al.* Quantitative brain sodium MRI depicts corticospinal impairment in amyotrophic lateral sclerosis. Radiology 2019; 292:422–428.
68. ■■ Christidi F, Karavasilis E, Argyropoulos GD, *et al.* Neurometabolic alterations in motor neuron disease: insights from magnetic resonance spectroscopy. J Integr Neurosci 2022; 21:87.
MRS is an underutilized and versatile imaging method in ALS.
69. Verma G, Woo JH, Chawla S, *et al.* Whole-brain analysis of amyotrophic lateral sclerosis by using echo-planar spectroscopic imaging. Radiology 2013; 267:851–857.
70. Stagg CJ, Knight S, Talbot K, *et al.* Whole-brain magnetic resonance spectroscopic imaging measures are related to disability in ALS. Neurology 2013; 80:610–615.
71. Proudfoot M, Bede P, Turner MR. Imaging cerebral activity in amyotrophic lateral sclerosis. Front Neurol 2018; 9:1148.
72. Westeneng HJ, Walhout R, Straathof M, *et al.* Widespread structural brain involvement in ALS is not limited to the C9orf72 repeat expansion. J Neurol Neurosurg Psychiatry 2016; 87:1354–1360.
73. Bede P, Hardiman O. Longitudinal structural changes in ALS: a three time-point imaging study of white and gray matter degeneration. Amyotroph Lateral Scler Frontotemporal Degener 2018; 19:232–241.

74. Bertrand A, Wen J, Rinaldi D, *et al.* Early cognitive, structural, and microstructural changes in presymptomatic C9orf72 carriers younger than 40 years. JAMA Neurol 2018; 75:236–245.

75. Chipika RH, Siah WF, McKenna MC, *et al.* The presymptomatic phase of amyotrophic lateral sclerosis: are we merely scratching the surface? J Neurol 2021; 268:4607–4629.

76. Popuri K, Dowds E, Beg MF, *et al.* Gray matter changes in asymptomatic C9orf72 and GRN mutation carriers. Neuroimage Clin 2018; 18:591–598.

77. Cash DM, Bocchetta M, Thomas DL, *et al.* Patterns of gray matter atrophy in genetic frontotemporal dementia: results from the GENFI study. Neurobiol Aging 2018; 62:191–196.

78. Gorges M, Vercruysse P, Müller HP, *et al.* Hypothalamic atrophy is related to body mass index and age at onset in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2017; 88:1033–1041.

79. Panman JL, Jiskoot LC, Bouts M, *et al.* Gray and white matter changes in presymptomatic genetic frontotemporal dementia: a longitudinal MRI study. Neurobiol Aging 2019; 76:115–124.

80. Ng MC, Ho JT, Ho SL, *et al.* Abnormal diffusion tensor in nonsymptomatic familial amyotrophic lateral sclerosis with a causative superoxide dismutase 1 mutation. J Magn Reson Imaging 2008; 27:8–13.

81. Turner MR, Hammers A, Al-Chalabi A, *et al.* Distinct cerebral lesions in sporadic and 'D90A' SOD1 ALS: studies with [11C]flumazenil PET. Brain 2005; 128:1323–1329.

82. Le Blanc G, Jetté Pomerleau V, McCarthy J, *et al.* Faster cortical thinning and surface area loss in presymptomatic and symptomatic C9orf72 repeat expansion adult carriers. Ann Neurol 2020; 88:113–122.

83. Walhout R, Schmidt R, Westeneng HJ, *et al.* Brain morphologic changes in asymptomatic C9orf72 repeat expansion carriers. Neurology 2015; 85:1780–1788.

84. Papma JM, Jiskoot LC, Panman JL, *et al.* Cognition and gray and white matter characteristics of presymptomatic C9orf72 repeat expansion. Neurology 2017; 89:1256–1264.

85. Lee SE, Sias AC, Mandelli ML, *et al.* Network degeneration and dysfunction in presymptomatic C9ORF72 expansion carriers. Neuroimage Clin 2017; 14:286–297.

86. Lulé DE, Müller HP, Finsel J, *et al.* Deficits in verbal fluency in presymptomatic C9orf72 mutation gene carriers: a developmental disorder. J Neurol Neurosurg Psychiatry 2020.

87. Tahedl M, Chipika RH, Lope J, *et al.* Cortical progression patterns in individual ALS patients across multiple timepoints: a mosaic-based approach for clinical use. J Neurol 2021; 268:1913–1926.

88. Bede P, Iyer PM, Finegan E, *et al.* Virtual brain biopsies in amyotrophic lateral sclerosis: diagnostic classification based on in vivo pathological patterns. Neuroimage Clin 2017; 15:653–658.

89. Schuster C, Hardiman O, Bede P. Development of an automated MRI-based diagnostic protocol for amyotrophic lateral sclerosis using disease-specific pathognomonic features: a quantitative disease-state Classification Study. PLoS One 2016; 11:e0167331.

90. Querin G, El Mendili MM, Bede P, *et al.* Multimodal spinal cord MRI offers accurate diagnostic classification in ALS. J Neurol Neurosurg Psychiatry 2018; 89:1220–1221.

91. Westeneng HJ, Debray TPA, Visser AE, *et al.* Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018; 17:423–433.

92. ■ Bede P, Chang KM, Tan EL. Machine-learning in motor neuron diseases: prospects and pitfalls. Eur J Neurol 2022; 29:2555–2256.
Machine-learning initiatives in ALS drive the transition from group-level studies to individual-patient data interpretation.

93. Muller HP, Turner MR, Grosskreutz J, *et al.* A large-scale multicentre cerebral diffusion tensor imaging study in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2016; 87:570–579.

94. Kassubek J, Muller HP, Del Tredici K, *et al.* Diffusion tensor imaging analysis of sequential spreading of disease in amyotrophic lateral sclerosis confirms patterns of TDP-43 pathology. Brain 2014; 137:1733–1740.

95. Bede P, Bogdahn U, Lope J, *et al.* Degenerative and regenerative processes in amyotrophic lateral sclerosis: motor reserve, adaptation and putative compensatory changes. Neural Regen Res 2021; 16:1208–1209.

96. ■ Bede P, Murad A, Lope J, *et al.* Clusters of anatomical disease-burden patterns in ALS: a data-driven approach confirms radiological subtypes. J Neurol 2022; 269:4404–4413.
Cluster analyses of large data set help to uncover small clinically or genetically homogenous phenotypes.

97. Costello E, Rooney J, Pinto-Grau M, *et al.* Cognitive reserve in amyotrophic lateral sclerosis (ALS): a population-based longitudinal study. J Neurol Neurosurg Psychiatry 2021; 92:460–465.

98. Müller HP, Del Tredici K, Lulé D, *et al.* In vivo histopathological staging in C9orf72-associated ALS: a tract of interest DTI study. Neuroimage Clin 2020; 27:102298.

99. Müller HP, Gorges M, Kassubek R, *et al.* Identical patterns of cortico-efferent tract involvement in primary lateral sclerosis and amyotrophic lateral sclerosis: a tract of interest-based MRI study. Neuroimage Clin 2018; 18:762–769.

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