In the past two decades, structural and functional neuroimaging findings have greatly modified longstanding notions regarding the pathophysiology of amyotrophic lateral sclerosis (ALS). Neuroimaging studies have shown that anatomical and functional lesions spread beyond precentral cortices and corticospinal tracts, to include the corpus callosum; frontal, sensory, and premotor cortices; thalamus; and midbrain. Both MRI and PET studies have shown early and diffuse loss of inhibitory cortical interneurons in the motor cortex (increased levels of functional connectivity and loss of GABAergic neurons, respectively) and diffuse gliosis in white-matter tracts. In ALS endophenotypes, neuroimaging has also shown a diverse spreading of lesions and a dissimilar impairment of functional and structural connections. A possible role of PET in the diagnosis of ALS has recently been proposed. However, most neuroimaging studies have pitfalls, such as a small number and poor clinical characterisation of patients, absence of adequate controls, and scarcity of longitudinal assessments. Studies involving international collaborations, standardised assessments, and large patient cohorts will overcome these shortcomings and provide further insight into the pathogenesis of ALS.

Introduction

Amyotrophic lateral sclerosis (ALS) is a disorder of adult life characterised by progressive degeneration of upper and lower motor neurons and the frontal cortex. The cause of ALS is still unknown and no disease-modifying treatments are available, apart from the anti-glutamatergic drug riluzole, which increases survival by about 2–3 months without affecting muscle strength.1 Up to 10% of patients with ALS inherit a genetic mutation, whereas the other 90% of cases occur sporadically in the population.2 The most common ALS-related genes in white populations are C9orf72, SOD1, TARDBP, and FUS, which account for about two-thirds of cases of familial ALS.3 Diagnosis of ALS is largely based on its clinical presentation, progression of symptoms, and exclusion of other diseases, supported by neurophysiological and neuroimaging examinations.4,5

There is an increasing awareness that ALS is a clinically and pathogenically heterogeneous disease.6 Several different phenotypes of ALS exist, ranging from pure upper motor neuron disease (primary lateral sclerosis) to pure lower motor neuron disease (progressive muscular atrophy), with several demographically (age and sex) and prognostically different intermediate forms (flail arm, flail leg, prevalent upper motor neuron, and bulbar ALS).7 Moreover, up to 50% of patients with ALS have cognitive deficits, again with a range of different clinical presentations, ranging from overt frontotemporal dementia (FTD) to cognitive impairment below the diagnostic threshold for FTD (pure executive, pure non-executive, or pure behavioural impairment).8 MRI, PET, and SPECT have been used variably in about 200 ALS studies (appendix). The contribution of imaging to the understanding of ALS cannot be overlooked, since it has enabled study of the brains of patients with ALS in vivo and, to a lesser extent, longitudinally. There are three main areas of neuroimaging research. First, anatomical and functional changes in ALS have been identified on structural (MRI) and functional (functional MRI [fMRI], PET, and SPECT) neuroimaging, including the spread of cortical and subcortical lesions. The extensive application of structural magnetic-resonance-based techniques (panel) has improved our understanding of ALS pathophysiology and the mechanisms underlying the progressive degenerative process. These findings have also given some insight into the dysfunction of local and distant neural circuits in the various phases of the disease. Second, MRI and radiotracers have been used to identify CNS alterations that could be used to improve ALS diagnostic accuracy with clinically useful sensitivity and specificity. Third, these techniques are being used to assess promising biomarkers of progression of motor and non-motor lesions, which will be used in both clinical (as markers of prognosis in a patient) and research settings (as biological markers for assessing the efficacy of experimental treatments).

Structural changes

Structural T1-weighted imaging

Structural T1-weighted MRI enables detailed analysis of focal brain atrophy, which is a key feature of patients with ALS. Cross-sectional voxel-based morphometry studies have yielded inconsistent results regarding the presence of atrophy in the primary motor cortex or premotor cortex in ALS and the extent of extra-motor atrophy, largely because of differences in sample sizes, image preprocessing, and statistical analysis, but also because of the clinical, cognitive, and genetic characteristics of patients (appendix). On the contrary, findings from studies of surface-based morphometry in ALS have revealed cortical thinning of the primary motor cortex.9–11 Furthermore, findings from large studies12,13 have shown that frontotemporal and parietal loss or thinning, which is seen in patients with ALS with normal cognitive functions, is more severe in patients with cognitive impairment and ALS with FTD than in those with only motor ALS.
Diffusion tensor MRI
Pathological abnormalities of ALS are a result of the degeneration of white-matter fibres, mainly the corticospinal tracts and corpus callosum. Diffusion tensor MRI studies of patients with ALS have consistently reported decreased fractional anisotropy and increased mean diffusivity of the corticospinal tract associated with greater disease severity and faster rate of disease progression; decreased fractional anisotropy of middle-posterior parts of the corpus callosum (figure 1); and decreased fractional anisotropy and increased mean diffusivity in extra-motor regions, especially fronto-temporal areas (appendix). These findings are from group comparisons with broad overlap of fractional anisotropy and mean diffusivity values between patients with ALS and controls at an individual level.

Magnetic resonance spectroscopy
In ALS, neuronal loss can be captured using ¹H-magnetic resonance spectroscopy. Findings from ¹H-magnetic resonance spectroscopy studies in ALS have shown that N-acetylaspartate (NAA) concentrations or NAA:creatine, NAA:choline, and NAA:(creatine plus choline) ratios are reduced in the primary motor cortex of patients with ALS, and NAA:creatine and NAA:choline ratios are reduced along the full length of the intracranial corticospinal tract and in the brainstem (appendix). Moreover, primary motor cortex NAA concentrations and ratios are associated with disease severity and progression. Finally, NAA concentrations and ratios are decreased in premotor regions, the primary sensory cortex, thalamus, basal ganglia, and extra-motor frontal and parietal areas.

Other potentially interesting but less consistently reported ¹H-magnetic resonance spectroscopy findings need to be confirmed in future studies. Higher glutamate-glutamine concentrations in the primary motor cortex and brainstem of patients with ALS compared with controls support the notion of excitotoxicity in ALS. With regard to signalling, an imbalance between excitatory (raised glutamate-glutamine) and inhibitory (reduced GABA) signalling seems to be important in the pathogenesis of ALS.

Spinal cord MRI
ALS is characterised by severe cervical cord damage caused by degeneration of the corticospinal tracts and loss of lower motor neurons. Imaging the spinal cord is challenging because of the spatial inhomogeneity of the magnetic field strength in this region, the small physical dimension of the spinal cord cross-sectional area, and respiratory and cardiac motion (appendix). Despite these technical difficulties, the recent development of sophisticated magnetic resonance coils and fast imaging techniques has led to improved capability to reliably study this structure. Analysis of diffusion tensor MRI parameters from individual cervical segments showed the largest differences in fractional anisotropy were at more distal cervical segments, supporting the dying back hypothesis of neurodegeneration in ALS—ie, early degeneration in ALS probably occurs in the distal spinal cord segments rather than in the brain.

Functional MRI
Since its development in 1992, functional MRI has rapidly become the most widely used neuroimaging method to assess human brain function in health and disease. Most functional MRI experiments are based on BOLD contrast imaging. The identification of patterns in the spontaneous fluctuations in the BOLD signal of functional MRI has been termed resting-state functional connectivity and might lead to the translation of functional MRI into clinical care. Indeed, unlike task-associated functional MRI, resting-state functional MRI does not need the administration of an external stimulus or task, which greatly simplifies its application in a clinical setting.

Panel: Quantitative magnetic resonance techniques applied in amyotrophic lateral sclerosis

Structural T1-weighted imaging
Automated and unbiased whole-brain analysis techniques segment and quantify grey-matter and white-matter morphology with T1-weighted images. Present analysis techniques include voxel-based and surface-based morphometry. Voxel-based morphometry allows the regional assessment of grey-matter or white-matter density. Surface-based morphometry reconstructs the boundaries between grey and white matter, and grey matter and CSF, allowing measurement of cortical thickness and surface area.

Diffusion tensor MRI
Diffusion tensor MRI can be used to map and characterise the three-dimensional diffusion of water as a function of spatial location. The two most common diffusion tensor MRI measures are mean diffusivity and fractional anisotropy. Mean diffusivity is a measure of the magnitude of diffusion and is rotationally invariant. Fractional anisotropy describes the amount of anisotropy (the property of being directionally dependent) of the diffusion tensor. The diffusion of water within the tissues will be altered by changes in the tissue microstructure and organisation caused by many pathological processes of the CNS, including demyelination, axonal damage, inflammation, oedema, and ischaemia.

¹H-magnetic resonance spectroscopy
¹H-magnetic resonance spectroscopy records signals from metabolites. Peaks in the conventional ¹H-magnetic resonance spectroscopy spectra correspond to different metabolites:

- Choline, a marker of cell membrane turnover
- Creatine, a marker of energy metabolism
- N-acetylaspartate (NAA), a marker of neuronal and axonal viability
- Lactate, which is normally not discernible from the baseline noise and should not be present in normal brain because it is produced by anaerobic metabolism
- Myo-inositol, which is localised within astrocytes and regarded as a glial marker
- Glutamate-glutamine, a marker of the glutamatergic neurotransmitter system

GABA, the major inhibitory neurotransmitter, is difficult to quantify using conventional ¹H-magnetic resonance spectroscopy, but can be measured using spectral editing techniques and other non-conventional methods.

Functional MRI
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BOLD=blood-oxygenation-level dependent.

Resting-state fMRI
Several resting-state fMRI studies of ALS reported significantly decreased functional connectivity within the sensorimotor network and in brain networks related to...
cognition and behaviour, in keeping with the altered motor and extramotor structural connectivity. Other studies have identified regions of increased functional connectivity, including somatosensory and extra-motor areas (figure 2; appendix). Two scenarios have been described to explain the increased functional connectivity in patients with ALS. First, functional connectivity might be increased to compensate for structural damage, but this increase is exhausted with increasing burden of pathology. This notion is supported by evidence that the pattern of increased functional connectivity of sensorimotor networks is more widespread when assessing only patients with ALS with preserved diffusion tensor MRI measures than when assessing patients with severe corticospinal tract damage, and that parietal connectivity is higher in the default mode network and in patients compared with healthy controls, in frontoparietal networks in patients with ALS compared with healthy controls, and that the...
clinical status and cognitive performance are better in ALS patients with higher parietal connectivity in the default mode network and frontoparietal networks than in healthy controls. Second, the high level of functional connectivity in ALS might be related to pathogenic loss of local inhibitory circuitry. Indeed, increased functional connectivity was found over a large area spanning sensorimotor, premotor, prefrontal, and thalamic regions that overlaps areas adjoining white-matter tracts, showing loss of integrity by diffusion tensor MRI (figure 2). In agreement with this second hypothesis, patients with primary lateral sclerosis with the highest functional connectivity within the sensorimotor, frontal, and left frontoparietal networks showed the greatest clinical disability, executive functional impairment, and white-matter tract microstructural damage (figure 2). This latter hypothesis is further supported by decreased GABA concentrations in the primary motor cortex of patients with ALS, as shown by $^1$H-magnetic resonance spectroscopy and PET investigations in which decreased uptake of $^{11}$C-flumazenil—a PET marker of benzodiazepine receptor unit distribution in pyramidal cells and inhibitory interneurons—was seen in the primary motor cortex and frontal cortex.

Most resting-state fMRI studies of ALS have focused on the sensorimotor network. Many other cortical systems that are potentially relevant to brain functional architecture remain to be explored.

**Task-associated fMRI**

In small samples of patients with ALS, compared with healthy controls fMRI tasks of limb movements have shown heightened biehmispheric activation of the primary motor cortex and premotor and supplementary motor areas, spatial shifts of recruitment to more anterior regions of the premotor cortex, activation of cerebral regions involved in motor learning (basal ganglia and cerebellum), and increased recruitment of extra-motor areas (eg, the temporal and inferior parietal cortices; appendix). Such changes might represent compensatory cortical plasticity in response to loss of pyramidal cells in the primary motor cortex or reduced local inhibitory interneuronal function.

The relation between altered brain activation during motor tasks and the amount of upper motor neuron involvement suggests that activity in the contralateral primary motor cortex decreases most as physical impairments worsen in patients with ALS. Accordingly, activation of extra-motor areas during motor tasks is lower in patients with faster disease progression over 1 year than in patients with slower disease progression.

Furthermore, task-associated fMRI data provide evidence for a multi-system involvement of cognitive and emotional processing pathways in ALS. Cerebral activation is altered when undertaking executive and language tasks and processing of socioemotional stimuli in patients with ALS who do not have dementia.

**Radiotracer imaging in ALS**

In the late 1980s, the association between ALS and dementia syndromes, specifically FTD, was recognised by neuropsychological investigations. PET and SPECT studies were undertaken to identify metabolic, blood flow, and, more recently, receptor changes associated with or responsible for clinical symptoms of these disorders (appendix).

Widespread decreased glucose metabolism in the cerebral cortex and basal ganglia was found in 1987 in the first $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) PET study in ALS and was later associated with disease duration, muscular weakness, and poor performance on the word fluency test (appendix). In ALS, cognitive changes in the frontal cortex were extensively investigated in the 1990s by $^{15}$O-PET, which showed overall attenuated responses to tasks involving executive functions (appendix). In cohorts of patients with ALS and cognitive impairment, the frontal and temporal lobes were hypometabolic and there were no accumulations of $\beta$-amyloid, which are typically absent in FTD, confirming the pathological similarities between these two diseases. Additionally, in patients with ALS compared with healthy controls or with other brain regions, in cerebral blood flow distribution studies performed by $^{123}$I-iodoamphetamine SPECT, $^{99m}$Tc-hexamethylpropylene amine oxide SPECT, or $^{99m}$Tc-ethyl-cysteinate dimer SPECT, perfusion was reduced in the frontal and, to a lesser extent, the motor cortex (appendix).

The extent of cerebral blood flow deficit in the motor, frontal, and anterior temporal cortices was associated with a high level of cognitive impairment, which was not present when cerebral blood flow decrease was confined to motor areas. Neuropsychological and perfusion impairments are more pronounced in patients with comorbid ALS and FTD and those with pure FTD than in those with ALS, which suggests a continuum between the two disorders.

Also, hypoperfusion in the frontal cortex was associated with impaired executive functions in patients with ALS, and severe neuropsychopathological features, such as spongiform degeneration and neuronal loss in the frontal lobe, were identified post mortem in patients in whom the cognitive disability was more pronounced.

Conversely, hypermetabolism was noted in the midbrain and medial temporal cortex of patients with ALS, particularly in patients with spinal onset (figure 3). Hypermetabolism was possibly related to the reactive astrocytosis that surrounds the affected motor neurons, the descending white-matter tracts fanning out from the primary motor cortex, and the brainstem, being astrocytes the main determinant of $^{18}$F-FDG uptake in the astrocyte-neuron functional unit. This is also consistent with findings that show increased upregulation of peripheral benzodiazepine receptors expressed by activated microglia, as shown by increased binding of $^{11}$C(R)PK11195 and $^{18}$F-DPA-714 in the sensorimotor cortex and midbrain, and increased astrocytosis, as...
shown by upregulation of monoamine oxidase B activity in the pons and subcortical white matter measured with $^{11}$C(L)-deprenyl-D2)\textsuperscript{(76)} (appendix). This notion is corroborated by the findings that myoinositol, a spectroscopic marker of glial activity, is increased in the primary motor cortex of patients with ALS\textsuperscript{(77)}.

Receptor studies of the integrity of neurons have been done by $^{11}$C-flumazenil-PET—a sensitive marker of benzodiazepine GABA-A receptor unit subtypes, which are widely distributed in pyramidal cells and inhibitory interneurons—in patients with sporadic ALS\textsuperscript{(32)}, patients with the homozygous Asp90Ala SOD1 mutation\textsuperscript{(78)} and patients with primary lateral sclerosis (appendix)\textsuperscript{(79,80)}. These studies reported reduced uptake in the motor and frontal cortex of patients with ALS and, in keeping with findings from fMRI studies, in addition to neuronal loss, disrupted GABAergic neurotransmission, with reduced inhibitory effects that extended to extra-motor areas. The loss of neurons in ALS was also confirmed by decreased uptake of a selective serotonin-1A receptor radioligand ($^{11}$C-WAY100635) in the motor and frontal cortex\textsuperscript{(81)}, possibly resulting in an absence of the inhibitory effect on pyramidal cell firing. This absence of inhibitory responses would contribute to glutamate excitotoxicity, supposedly one of the pathogenic mechanisms of ALS. Reduced $^{11}$C-flumazenil uptake was also associated with deficits in word generation\textsuperscript{(82)} and writing errors\textsuperscript{(83)}, highlighting the possible role of $^{11}$C-flumazenil in studies of cognition. In presynaptic dopaminergic studies, an $^{18}$F-fluorodopa deficit was noted in patients with sporadic ALS\textsuperscript{(84,85)} which was associated with disease duration (appendix)\textsuperscript{(86)}. Impaired neuronal integrity has also been shown by reduced $^{18}$F-fluoro-propyl-carbomethoxy-3β-(4-iodophenyl) nortropane transporter uptake in patients with comorbid ALS and Parkinson’s disease\textsuperscript{(77)}, suggesting some overlap between the two disorders.

Findings from functional studies in ALS suggest a multisystemic model of the disease, in which compensatory plasticity and cognitive deficits affect brain areas beyond the motor regions, and suggest possible preferential anatomo-functional ways of disease spread.

Clinical associations

Neuroimaging as a diagnostic marker of ALS

In patients with ALS, conventional MRI is frequently not informative and its diagnostic use is restricted to exclusion of other mimic disorders.\textsuperscript{(3,4)} Although the detection of corticospinal tract hyperintensities on conventional MRI and the presence of a T2-hypointense rim in the primary motor cortex can support a pre-existing suspicion of ALS, they are neither sensitive nor specific for ALS and are not recommended for a firm diagnosis.\textsuperscript{(88)}

Findings from some studies suggested cortical thinning of the primary motor cortex as a sensitive diagnostic marker at the individual patient level\textsuperscript{(10,89)}. In a meta-analysis\textsuperscript{(90)} of studies of diffusion tensor MRI diagnostic accuracy in ALS, pooled sensitivity was 65% and pooled specificity was 67%. In a large ALS population\textsuperscript{(91)}, the NAA to creatine ratio had high sensitivity (86%) but low specificity (37%) to detect upper motor neuron signs. The myo-inositol to NAA ratio has a better sensitivity (71%) and specificity (93%) profile than NAA to creatine and choline ratios.\textsuperscript{(77)} The combination of $^1$H-magnetic resonance spectroscopy with transcranial magnetic stimulation or diffusion tensor MRI showed potential to improve sensitivity of detection of upper motor neuron degeneration.\textsuperscript{(91,92)} Although resting-state fMRI studies in ALS were done at a group level, preliminary data on sensitivity and specificity as assessed by machine learning are promising\textsuperscript{(25,93)}.

$^{18}$F-FDG-PET is potentially an important biomarker for diagnosis of ALS, as suggested by two recent investigations including 195 and 70 patients\textsuperscript{(63,94)}, the largest PET cohorts of patients with ALS so far. In addition to the confirmation of motor and extra-motor hypometabolism (figure 4)\textsuperscript{(74,95)}, and hypermetabolism in...
the brainstem and medial temporal cortex, both investigations resulted in an overall accuracy for \(^{18}\text{F-FDG-PET}\) in discriminating patients with ALS from healthy controls of about 93%.

**Neuroimaging as a marker of ALS phenotypic and genetic variants**

Evidence is mounting regarding different patterns of white-matter damage in specific groups of patients with ALS or other motor neuron diseases. For example, in patients with primary lateral sclerosis, the most severe abnormalities occur in callosal motor fibres—a common pathway in upper motor neuron variants (figure 1).\(^{14,15}\) Diffuse white-matter damage has been associated with cognitive deficits in ALS: performance in cognitive tests that assess attention and executive functions was associated with diffusion tensor MRI changes in the corpus callosum, corticospinal tract, and long association tracts.\(^{16,17}\) Also, decreased frontal NAA to creatine ratio\(^{18,19}\) and metabolism on PET\(^{20}\) is associated with executive function deficits, underscoring the involvement of extra-motor regions in ALS. Conversely, hypermetabolism due to astrocytosis has been detected in the corticospinal tract\(^{21,22}\) and can be potentially regarded as a disease hallmark. ALS is the only neurodegenerative disorder in which there is white-matter hypermetabolism, and that might be helpful in discriminating between patients with ALS and healthy controls or ALS-mimicking syndromes.

Only a few studies so far have used voxel-based morphometry or surface-based morphometry, or both, to characterise the pattern of grey-matter loss in patients with specific ALS genotypes,\(^{100,101}\) including patients homozygous for the Asp90Ala SOD1 mutation and those with the C9orf72 repeat expansion. In patients with the C9orf72 repeat expansion, substantial extra-motor cortical atrophy occurs (figure 5).\(^{100}\) ALS caused by genetic mutations might show specific patterns of white-matter damage. In a study of presymptomatic SOD1 mutation carriers, structural white-matter changes occurred before symptom onset.\(^{102}\) Patients with familial ALS who carry Asp90Ala SOD1 mutations had less severe white-matter abnormalities than did patients with sporadic ALS with a similar level of disease severity.\(^{103,104}\) Findings from a pivotal \(^{1}H\)-magnetic resonance spectroscopy study of presymptomatic carriers of SOD1 mutations showed reduced metabolite ratios in the cervical cord, which were similar to those in patients with ALS.\(^{105}\)

30 patients with sporadic ALS were also compared by PET with groups of patients with comorbid ALS and FTD and patients with ALS carrying the C9orf72 mutation;\(^{106}\) in those with the C9orf72 mutation, there was a more widespread cortical and subcortical involvement along with more severe clinical course. Extensive fronto-temporal involvement was identified in association with the C9orf72 genotype.\(^{106}\)

**Neuroimaging as a marker of disease progression**

Clinical outcome is determined by the interaction of functional changes with structural damage. Structural neuroimaging may have a role in identifying potential biomarkers of disease progression, in providing a complete picture of the involvement of extra-motor brain regions, and in understanding how functional changes are associated with structural damage to determine clinical outcomes.

**Structural T1-weighted imaging**

Four longitudinal studies with small patient samples assessed the development of brain atrophy or thinning in
ALS (appendix). Progressive damage occurred in the primary motor cortex over 9–12 months, although this finding was not confirmed in other studies. Additionally, progressive atrophy or thinning of extra-motor regions took place in patients with ALS over 3–9 months. The relations between clinical signs and symptoms or volume or thickness changes were not clear. Progression of disability over 1 year associated with primary motor cortex thinning was reported in one study. In two studies, primary motor cortex and temporal lobe atrophy was accelerated in patients with more rapidly progressive disease, but this finding was not confirmed in another study. Finally, a faster rate of cortical thinning was reported in patients with ALS with shorter disease duration, suggesting that thickness might decrease in a non-linear fashion.

**Diffusion tensor MRI**

Diffusion tensor MRI might predict clinical evolution. Findings from two studies showed a prognostic value of corticospinal tract diffusion tensor MRI in patients with ALS (appendix). Ten longitudinal studies used diffusion tensor MRI to track the evolution of brain white-matter changes in sporadic ALS. A substantial progression of brain corticospinal tract damage after 6 months was reported in some studies, but not in others. Findings from two studies showed a decrease of whole brain or corticospinal tract fractional anisotropy after 8 months. The relation between brain diffusion tensor MRI changes and worsening disability in these patients was significant in only two of four studies. One study reported diffusion tensor MRI changes after 6 months in the corticospinal tract, corpus callosum, and several frontal and temporal regions in progressive muscular atrophy. Power calculations were done for two longitudinal studies that investigated changes in corticospinal tract fractional anisotropy for 8 months in one study calculation, 46 patients were needed to detect significant changes in fractional anisotropy (80% power and alpha level of 0·05). In the other, a sample size of 263 patients per arm was needed to detect a 25% treatment effect on fractional anisotropy in patients with ALS. The levels of recruitment were not met in either study. Longitudinal diffusion tensor MRI studies did not include cognitive and genetic assessments of patients and controls; only two longitudinal studies compared changes in patients with those in healthy participants, accounting for possible ageing effects.

**Magnetic resonance spectroscopy**

12 reports on natural history or pharmacology used ¹H-magnetic resonance spectroscopy to assess metabolic brain changes in ALS (appendix). Reductions in NAA concentrations and NAA to creatine and choline ratios in the primary motor cortex have been reported over follow-up periods of up to 28 months in six studies; however, one study reported no changes after 6–15 months. Findings from two studies suggested that changes in NAA concentrations are associated with disease progression. ¹H-magnetic resonance spectroscopy has been used to monitor the therapeutic effects of riluzole, creatine, and minocycline on brain metabolites in small cohorts of patients with ALS. The absence of an untreated patient arm in all studies to monitor therapeutic effects limits the interpretation of these findings.

**Spinal cord MRI**

Preliminary studies reported atrophy of the cervical cord in patients with ALS which progressed over a 9-month period, but this finding was not confirmed in another study (appendix). Diffusion tensor MRI has been successfully used to grade the extent of cervical cord damage associated with ALS. An association was found between cervical cord fractional anisotropy and measures of disease severity. Forced vital capacity, and mean finger and foot tapping speed. After 9 months, patients with ALS showed a significant decrease of cord fractional anisotropy and a significant increase of cord mean diffusivity. Reduced NAA to creatine and myo-inositol ratios in the cervical cord are associated with Amyotrophic Lateral Sclerosis Functional Rating Scale score and reduced forced vital capacity in ALS.

**Pitfalls of MRI and radiotracer imaging studies**

Despite great achievements, most published studies have several pitfalls (table). Some of the differences in reported results might be because of clinical and demographic characteristics of patients, particularly the variable duration of the disease at the time of MRI and PET or SPECT (ranging from 6 months to 180 months)—ie, patients are at different stages of the disease. Neurobiological evidence that the different involvement of cortical bulbar and spinal motor neurons from an early stage in ALS represents different endophenotypes of the disorder, and suggests that there is need to recruit clinically homogeneous groups of patients in neuroimaging studies.

More importantly, there are relatively few longitudinal studies with MRI and none with PET or SPECT, and those studies that have been done have several shortcomings, regardless of the MRI technique. These shortcomings include small sample sizes, absence of a power calculation, and a high attrition rate because of the difficulty in undertaking longitudinal MRI assessments as bulbar and respiratory weakness worsens. Moreover, published longitudinal MRI studies include a selection of patients whose disease course has stabilised, allowing long scans to be done. Therefore, these studies show only small changes on MRI because of the slow progress of the advanced disease course. Finally, MRI changes are not adjusted for the duration of follow-up and for disease severity at baseline in some studies, and no reproducibility data are provided. With only one exception, longitudinal structural MRI studies are also characterised by the absence of comprehensive neuropsychological assess-
Evolving imaging techniques that allow the assessment of functional changes.

**Conclusions and future directions**

MRI and radiotracer imaging are powerful and rapidly evolving imaging techniques that allow the assessment of the involvement of brain structures and functions in ALS in vivo. Although conceptually and methodologically different, their findings tend to be concordant (figure 6). There is substantial anatomical and functional damage of the primary motor cortex, associated with damage to the corpus callosum (in particular, the middle and posterior parts). The degeneration of corticospinal tracts and the associated gliosis is shown by both MRI and PET. There is a diffusion of anatomical and functional lesions beyond the classic areas, including not only to the frontal cortex (along with cognitive deficits), but also to sensorimotor, premotor, thalamic, and midbrain regions. Furthermore, there is a diffuse and probably

<table>
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<tr>
<th>General caveats</th>
<th>Solutions</th>
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<tbody>
<tr>
<td>Most studies include a small number of participants (eg, of about 150 studies on MRI, only ten included more than 50 patients and the median number of cases included was 25). The number of patients included in PET and SPECT studies is even lower (median number of cases included was 12 and only seven of 40 studies included more than 20 cases).</td>
<td>Increase the number of patients in each study; undertake studies combining cohorts of patients from different ALS centres.</td>
</tr>
<tr>
<td>Poor clinical characterisation of patients: only age, sex, site of onset, and disease duration are usually reported; in several studies, cognitive status is also described, whereas other phenotypic characteristics are never reported.</td>
<td>Provide a detailed description of patients’ clinical phenotype, including extent of upper and lower motor neuron involvement, clinical subtypes, and neurophysiological data.</td>
</tr>
<tr>
<td>Scarcity of adequate healthy controls</td>
<td>How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
</tr>
<tr>
<td>Scarcity of disease controls (only seven studies have compared patients with ALS with those with an ALS mimic disorder and only one study has compared patients with ALS with those with pure frontotemporal dementia)</td>
<td>Patients with ALS should be compared with patients with other disorders of the motor neuron spectrum, involving both the CNS and the peripheral nervous system, to improve the diagnostic yield of MRI and PET. Patients with ALS should be compared with patients with cognitive impairment but without motor involvement (pure frontotemporal dementia) to gain more information on the pathological process of both disorders. The asymmetry of changes, if any, should be assessed according to the different amounts of cognitive impairment in patients with ALS.</td>
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<tr>
<td>Limited investigation of the laterality of lesions</td>
<td>Progressive changes in PET scans have been observed in patients with ALS. How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
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<tr>
<td>Scarcity of genotyping, with the exception of patients with C9orf72 mutations and homozygous Asp90Ala SOD1 mutations</td>
<td>Patients with mutations of other ALS-related genes and those with SOD1 mutations different from Asp90Ala should be studied to better elucidate the pathological process of ALS. MRI and PET findings should be compared with rating scales for upper motor neuron function. Comparisons should also be made with neurophysiological measures of upper motor neuron involvement.</td>
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<td>Association with functional scores that are not relevant for the examined areas (ie, association of cortical lesions with the revised version of the ALS Functional Rating Scale or with measures of muscle function)</td>
<td>Improve statistical analysis methodology by calculating the sample power, taking into account possible confounders such as age, sex, and education, and correcting for multiple comparisons.</td>
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<tr>
<td>Suboptimum statistical analyses (ie, absence of correction for multiple comparisons, low statistical thresholds)</td>
<td>A direct comparison between MRI and PET in the same cohort could give useful information about the nature of the brain changes, possibly by new PET-MRI methodology.</td>
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<tr>
<td>Absence of studies comparing findings from MRI and PET in the same cohort of patients</td>
<td>Include technical and procedural details of the imaging method. Use standardised methods to acquire and process data.</td>
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<tr>
<td>Poor description of the scanning protocol and high protocol variability among studies (eg, variability in magnet field strength, MRI equipment, acquisition sequence, and analytical approaches)</td>
<td>Use new diffusion imaging methods, such as high angular diffusion imaging and Q-ball imaging, to obtain more accurate depictions of intersecting tracts, as suggested by preliminary studies in ALS.</td>
</tr>
<tr>
<td>Technical caveats</td>
<td>Acquire 1H-magnetic resonance spectroscopy data using 3T magnetic resonance scanners.</td>
</tr>
<tr>
<td>Diffusion tensor MRI: crossing white-matter pathways (eg, intersecting pathways in the centrum semiovale), because most tractography algorithms are unable to resolve crossing white-matter pathways</td>
<td>Resting-state functional MRI needs no tasks and places little demand on patients.</td>
</tr>
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<td>1H-magnetic resonance spectroscopy is more susceptible than other magnetic resonance techniques to lower fields caused by a reduction of both signal-to-noise ratio and spectral resolution</td>
<td>New PET and MRI systems or PET scanners with higher spatial resolution along with software merging of MRI and PET data from the same patient will allow precise anatomical localisation of the signal.</td>
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<tr>
<td>Task-associated functional MRI: slight patient movement during a task-associated functional MRI scan can lead to increased signal variance; the choice and standardisation of the stimulation task, and the large variability between individuals and session, might bias the results</td>
<td>Higher sensitivity of the new-generation PET scanners will allow examination with lower radiation doses.</td>
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<td>The low spatial resolution of PET and SPECT can result in a difficult assessment of radiotracer uptake in small structures and in the smearing of signal from grey to white matter and vice versa</td>
<td>Provide a detailed description of patients’ clinical phenotype, including extent of upper and lower motor neuron involvement, clinical subtypes, and neurophysiological data.</td>
</tr>
<tr>
<td>Longitudinal PET and SPECT studies involving healthy controls are difficult because of restrictions in dosimetry regulations</td>
<td>How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
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### Table: Major caveats in MRI and radionuclide imaging studies of amyotrophic lateral sclerosis and their possible solutions

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<th>Caveats</th>
<th>Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most studies include a small number of participants (eg, of about 150 studies on MRI, only ten included more than 50 patients and the median number of cases included was 25). The number of patients included in PET and SPECT studies is even lower (median number of cases included was 12 and only seven of 40 studies included more than 20 cases).</td>
<td>Increase the number of patients in each study; undertake studies combining cohorts of patients from different ALS centres.</td>
</tr>
<tr>
<td>Poor clinical characterisation of patients: only age, sex, site of onset, and disease duration are usually reported; in several studies, cognitive status is also described, whereas other phenotypic characteristics are never reported.</td>
<td>Provide a detailed description of patients’ clinical phenotype, including extent of upper and lower motor neuron involvement, clinical subtypes, and neurophysiological data.</td>
</tr>
<tr>
<td>Scarcity of adequate healthy controls</td>
<td>How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
</tr>
<tr>
<td>Scarcity of disease controls (only seven studies have compared patients with ALS with those with an ALS mimic disorder and only one study has compared patients with ALS with those with pure frontotemporal dementia)</td>
<td>Patients with ALS should be compared with patients with other disorders of the motor neuron spectrum, involving both the CNS and the peripheral nervous system, to improve the diagnostic yield of MRI and PET. Patients with ALS should be compared with patients with cognitive impairment but without motor involvement (pure frontotemporal dementia) to gain more information on the pathological process of both disorders. The asymmetry of changes, if any, should be assessed according to the different amounts of cognitive impairment in patients with ALS.</td>
</tr>
<tr>
<td>Limited investigation of the laterality of lesions</td>
<td>Progressive changes in PET scans have been observed in patients with ALS. How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
</tr>
<tr>
<td>Scarcity of genotyping, with the exception of patients with C9orf72 mutations and homozygous Asp90Ala SOD1 mutations</td>
<td>Patients with mutations of other ALS-related genes and those with SOD1 mutations different from Asp90Ala should be studied to better elucidate the pathological process of ALS. MRI and PET findings should be compared with rating scales for upper motor neuron function. Comparisons should also be made with neurophysiological measures of upper motor neuron involvement.</td>
</tr>
<tr>
<td>Association with functional scores that are not relevant for the examined areas (ie, association of cortical lesions with the revised version of the ALS Functional Rating Scale or with measures of muscle function)</td>
<td>Improve statistical analysis methodology by calculating the sample power, taking into account possible confounders such as age, sex, and education, and correcting for multiple comparisons.</td>
</tr>
<tr>
<td>Suboptimum statistical analyses (ie, absence of correction for multiple comparisons, low statistical thresholds)</td>
<td>A direct comparison between MRI and PET in the same cohort could give useful information about the nature of the brain changes, possibly by new PET-MRI methodology.</td>
</tr>
<tr>
<td>Absence of studies comparing findings from MRI and PET in the same cohort of patients</td>
<td>Include technical and procedural details of the imaging method. Use standardised methods to acquire and process data.</td>
</tr>
<tr>
<td>Poor description of the scanning protocol and high protocol variability among studies (eg, variability in magnet field strength, MRI equipment, acquisition sequence, and analytical approaches)</td>
<td>Use new diffusion imaging methods, such as high angular diffusion imaging and Q-ball imaging, to obtain more accurate depictions of intersecting tracts, as suggested by preliminary studies in ALS.</td>
</tr>
<tr>
<td>Technical caveats</td>
<td>Acquire 1H-magnetic resonance spectroscopy data using 3T magnetic resonance scanners.</td>
</tr>
<tr>
<td>Diffusion tensor MRI: crossing white-matter pathways (eg, intersecting pathways in the centrum semiovale), because most tractography algorithms are unable to resolve crossing white-matter pathways</td>
<td>Resting-state functional MRI needs no tasks and places little demand on patients.</td>
</tr>
<tr>
<td>1H-magnetic resonance spectroscopy is more susceptible than other magnetic resonance techniques to lower fields caused by a reduction of both signal-to-noise ratio and spectral resolution</td>
<td>New PET and MRI systems or PET scanners with higher spatial resolution along with software merging of MRI and PET data from the same patient will allow precise anatomical localisation of the signal.</td>
</tr>
<tr>
<td>Task-associated functional MRI: slight patient movement during a task-associated functional MRI scan can lead to increased signal variance; the choice and standardisation of the stimulation task, and the large variability between individuals and session, might bias the results</td>
<td>Higher sensitivity of the new-generation PET scanners will allow examination with lower radiation doses.</td>
</tr>
<tr>
<td>The low spatial resolution of PET and SPECT can result in a difficult assessment of radiotracer uptake in small structures and in the smearing of signal from grey to white matter and vice versa</td>
<td>Provide a detailed description of patients’ clinical phenotype, including extent of upper and lower motor neuron involvement, clinical subtypes, and neurophysiological data.</td>
</tr>
<tr>
<td>Longitudinal PET and SPECT studies involving healthy controls are difficult because of restrictions in dosimetry regulations</td>
<td>How controls have been selected should be reported in detail. Besides matching for age and sex, population-based controls would be necessary to avoid selection bias. Finally, a detailed clinical characterisation of controls, including their cognitive status, should be reported.</td>
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</tbody>
</table>

ALS=amyotrophic lateral sclerosis.
early loss of inhibitory cortical interneurons shown by both fMRI (increased levels of functional connectivity) and ¹¹C-flumazenil PET (loss of GABAergic inhibitory neurons in the motor cortex). Patterns of lesions differ between patients with ALS and those patients with primary lateral sclerosis, suggesting that ALS endophenotypes are characterised by diverse spreading of lesions and, possibly, by different impairment of functional connections. A specific pattern of lesion distribution in patients carrying the C9orf72 mutation has been identified, which is characterised by widespread non-motor cortical and subcortical involvement.

A possible diagnostic role of MRI and, more recently, FDG-PET in the diagnosis of ALS is emerging, although it should be confirmed by further studies. Finally, several MRI longitudinal studies have pointed out possible markers of disease progression, such as atrophy or thinning of motor and extra-motor regions and corticospinal tract damage over time, but more data are needed to translate these findings to clinical practice. Future prospective studies should include larger series of patients, ideally recruited shortly after diagnosis, with longitudinal examinations for at least 12 months—to identify significant changes—and a multimodal (structural and functional) neuroimaging assessment. In this respect, novel PET-MRI technology might have a pivotal role in providing a patient-friendly and simultaneous assessment of the progressive structural and functional spread of lesions.

Functional studies with PET and SPECT can identify synaptic dysfunction and gliosis early in the course of the disease, before atrophy results from extending pathological changes. Specific endophenotypes could be identified in different clinical presentations (ie, spinal vs bulbar) and genetic variants. This identification of endophenotypes might be achieved using radiotracers for glucose metabolism, inflammation, or receptor density and might help to stratify patients for prognosis and hopefully treatment. Lastly, the promising role of PET as a
diagnostic biomarker should be further explored in longitudinal studies.

Unbiased, whole-brain 3T ¹H-magnetic resonance spectroscopy sequence and automated processing software offer potential for this technique as a biomarker of ALS in multicentre and longitudinal studies. However, coordinated implementation and centralised analysis of data are imperative if outcome measures are to be produced with sufficient reliability.

The Neuroimaging Society in ALS represents a major effort in this direction and will probably enable collection of data from a large number of patients, with different phenotypes and genetic backgrounds, which would provide the basis for the ultimate translation of ALS neuroimaging findings into clinical settings.

Neuroimaging also offers the possibility to undertake cross-sectional and longitudinal preclinical studies on people with mutations of ALS-related genes who are therefore at risk of developing ALS. These studies would provide a unique possibility to measure the spread of lesions in the preclinical phase, up to the development of motor and cognitive symptoms.

Finally, MRI and radiotracer imaging will enable stratification of patients according to their intrinsic progression rate, thus optimising disease management, improving the cost-effectiveness of care, enhancing the design of drug trials, and guiding the use of individualised treatments when these become available.

**Contributors**

ACH, MP, and MF conceived, designed, and supervised the study. ACh, MP, FA, AcA, AcC, and MF did the literature search, analysed and interpreted data, and critically revised the manuscript. ACh, MP, FA, and MF drafted the manuscript. ACh and MF obtained funding.

**Declaration of interests**

ACH has served on scientific advisory boards for Biogen Idec, Cytokinetics, and Italfarmaco; and has received research support from the Italian Ministry of Health, Italian Ministry of Education University and Research, European Communities, Regione Piemonte, Compagnia di San Paolo, Agenzia Italiana per la Ricerca sulla SLA (ARISLA), Fondazione Vialli e Mauro Onlus, and Federazione Italiana Giutoo Calci (FiGic). MP has received speaker honoraria from Piramal. FA has received speaker honoraria from Bayer Schering Pharma, Biogen, Sanofi-Aventis, and Serno Symposia International Foundation; and receives research support from the Italian Ministry of Health and ArisLA (Fondazione Italiana di Ricerca per la SLA). ACA receives research support from the Italian Ministry of Health and Regione Piemonte. ACA is employed by Positron Emission Tomography Centre IRMET Spa, Euromedic Inc. MF serves on scientific advisory boards for Teva Pharmaceutical Industries and Genmab; has received compensation for consulting services or speaking activities from Bayer Schering Pharma, Biogen Idec, Genmab, Merck Serono, and Teva Pharmaceutical Industries; and receives research support from Bayer Schering Pharma, Biogen Idec, Genmab, Merck Serono, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, the Jacques and Gloria Gossweiler Foundation (Switzerland), and the Alzheimer’s Disease Discovery Foundation.

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