Journal of Integrative Neuroscience

J. Integr. Neurosci. **2022**; 21(3): 87
https://doi.org/10.31083/j.jin2103087

Review

Neurometabolic Alterations in Motor Neuron Disease: Insights from Magnetic Resonance Spectroscopy

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Academic Editor: Luis Puelles
Submitted: 14 February 2022 Revised: 30 March 2022 Accepted: 15 April 2022 Published: 24 April 2022

Abstract

Magnetic resonance spectroscopy (MRS) has contributed important academic insights in motor neuron diseases (MNDs), particularly in amyotrophic lateral sclerosis (ALS). Over the past three decades momentous methodological advances took place, including the emergence of high-field magnetic resonance imaging (MRI) platforms, multi-voxel techniques, whole-brain protocols, novel head-coil designs, and a multitude of open-source imaging suites. Technological advances in MRS are complemented by important conceptual developments in MND, such as the recognition of the importance of extra-motor brain regions, multi-timepoint longitudinal study designs, assessment of asymptomatic mutation carriers, description of genotype-associated signatures, and the gradual characterisation of non-ALS MND phenotypes. We have conducted a systematic review of published MRS studies in MND to identify important emerging research trends, key lessons from pioneering studies, and stereotyped shortcomings. We also sought to highlight notable gaps in the current literature so that research priorities for future studies can be outlined. While MRS remains relatively underutilised in MND compared to other structural, diffusivity and functional imaging modalities, our review suggests that MRS can not only advance our academic understanding of MND biology, but has a multitude of practical benefits for clinical and pharmaceutical trial applications.

Keywords: magnetic resonance spectroscopy; amyotrophic lateral sclerosis; motor neuron disease; biomarkers; clinical trials

1. Introduction

Motor Neuron Diseases (MNDs) are a heterogeneous group of neurodegenerative conditions with divergent disability profiles [1], survival characteristics [2] and pathological patterns [3,4]. Imaging studies in MNDs are dominated by structural [5,6], diffusion [7] and fMRI studies [8,9], despite landmark magnetic resonance spectroscopy (MRS) studies, confirming medication effects [10], detecting presymptomatic changes [11], describing alterations in cortical [12] and subcortical [13] regions, in cross-sectional and longitudinal studies [14,15].

Complementary to structural gray matter (GM) and white matter (WM) magnetic resonance imaging (MRI), MRS allows non-invasive and *in vivo* quantification of different molecular constituents' (metabolites) concentration in brain regions based on their characteristic frequencies resonance in strong magnetic fields [16]. Even though earlier studies focused on the 31P nucleus for the measurement of energy metabolites (e.g., phosphocreatine and ATP) [17], the vast majority of brain MRS *in vivo* studies use proton MRS (1H-MRS) which became prevalent due its higher sensitivity and convenience in being performed on most clinical MRI machines [18]. 1H-MRS detects radiofrequency signals arising from hydrogen nuclear spins within tissue metabolites; these signals have chemically specific frequencies which are determined by the chemical environment of the hydrogen spins. The MRS signals are separated in the MR spectrum along chemical lines, i.e., chemical shift dimension. The spectrum is a plot of signal intensity (proportional to metabolite concentration) against the frequencies of resonance. The latter is reported in field-independent units, i.e., parts per million of the proton frequency (ppm). The most common metabolites that can be observed using brain 1H-MRS are lactate (Lac), lipids, alanine, N-acetyl-asparate (NAA), creatine

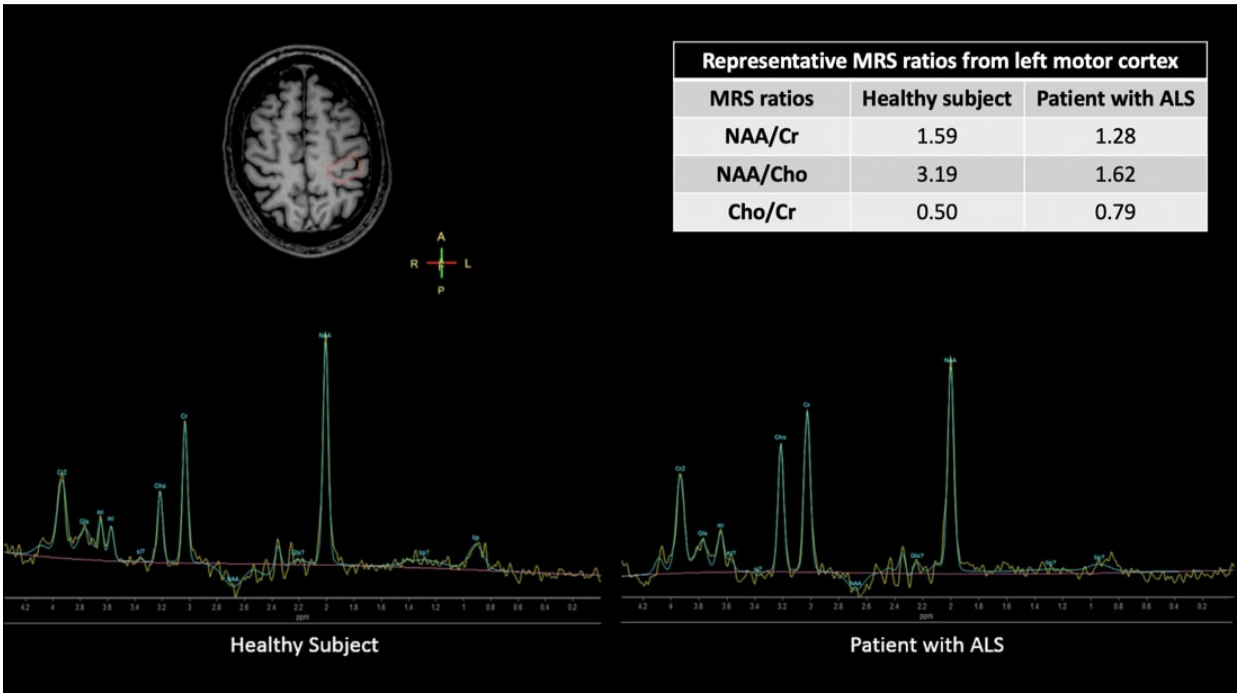

Fig. 1. The ^{1}H-MRS chemical shift of primary motor cortex at 3T for a healthy individual (left) and a patient with ALS (right). Representative spectra and corresponding ratios were acquired with single-voxel MRS scan (PRESS technique with water suppression at TE = 35 ms and TR = 2000 ms using a 2 × 2 × 1.5 cm voxel placed at the left motor cortex) from a right-handed healthy individual (left) and a right-handed patient with ALS (right). The y-axis corresponds to the detected signal intensity of metabolites in arbitrary units (i.e., relative metabolite concentrations), while the x-axis represents the frequency of chemical shift in parts per million (ppm) upon which metabolites are detected. The NAA/Cr and NAA/Cho ratios are reduced while the Cho/Cr is increased in the ALS-associated spectrum compared to the control one. MRS, magnetic resonance spectroscopy; PRESS, point-resolved spectroscopy sequence; TE, echo time; TR, repetition time; ms, milliseconds; cm, centimetres; NAA, N-acetylasparate; Cr, creatine; Cho, choline; mIns, myo-inositol; lip, lipids; Glx, Glutamate + Glutamine. Acquired spectra were obtained as part of a research MRS study which was approved by the local ethical committee of Aeginition Hospital; National and Kapodistrian Univeristy of Athens; GR (ΑΔΑ, ΨΔ4846Ψ8 Ν2 − Γ9Φ/06-11-2020); all participants provided informed consent before MRI scanning.

(Cr), choline (Cho), myo-inositol (mIns), glutamate (Glu), glutamine (Gln), and γ-aminobutyric acid (GABA). The combination of Glu and Gln is usually referred as Glx and is commonly reported instead of separate Glu and Gln due to the overlap of Glu and Gln signal at clinical field strengths [19]. On the other hand, GABA measurement is challenging due to overlap between its resonance and those of Cr and macromolecules and requires special techniques and higher magnetic field strength [20]. In general, the number of quantifiable metabolites depends on the magnetic field strength, chose pulse sequence and parameters, spectral resolution, signal to noise ratio (SNR), quality of B0 field homogeneity, and radiofrequency coil used [21]. The *in vivo* spatial localized metabolic information can be obtained using two methods: single-voxel spectroscopy uses selective excitatory pulses to localize a single voxel in the brain whereas magnetic resonance spectroscopic imaging (MRSI) is obtained in two or three dimensions allowing to collect the spectral information from a volume consisting of many voxels. MRS may have an important impact on patients' management providing major or complementary information for diagnosis, disease progression, monitoring therapeutic treatment and understanding disease pathogenesis [22]. Fig. 1 shows representative single-voxel ^{1}H MR spectra, along with relative concentrations of identifiable metabolites and corresponding ratios, acquired from the primary motor cortex of a healthy individual and a patient with ALS.

MND is a clinically heterogenous, inexorably progressive neurodegenerative condition. The term MND is an umbrella term which encompasses a multitude of phenotypes with unique clinical features, distinctive disability profiles, divergent progression rates and different survival profiles [3,4,23]. Pharmaceutical trials in MND have overwhelming targeted ALS cohorts to data and often rely on clinical scales, respiratory function and survival as study end-points instead of quantitative biomarkers [24–26], including MRS. Available MRS studies in ALS are mainly focus on motor areas and report reduced NAA in regions spanned by the pyramidal tract while few extra-motor MRS studies provide

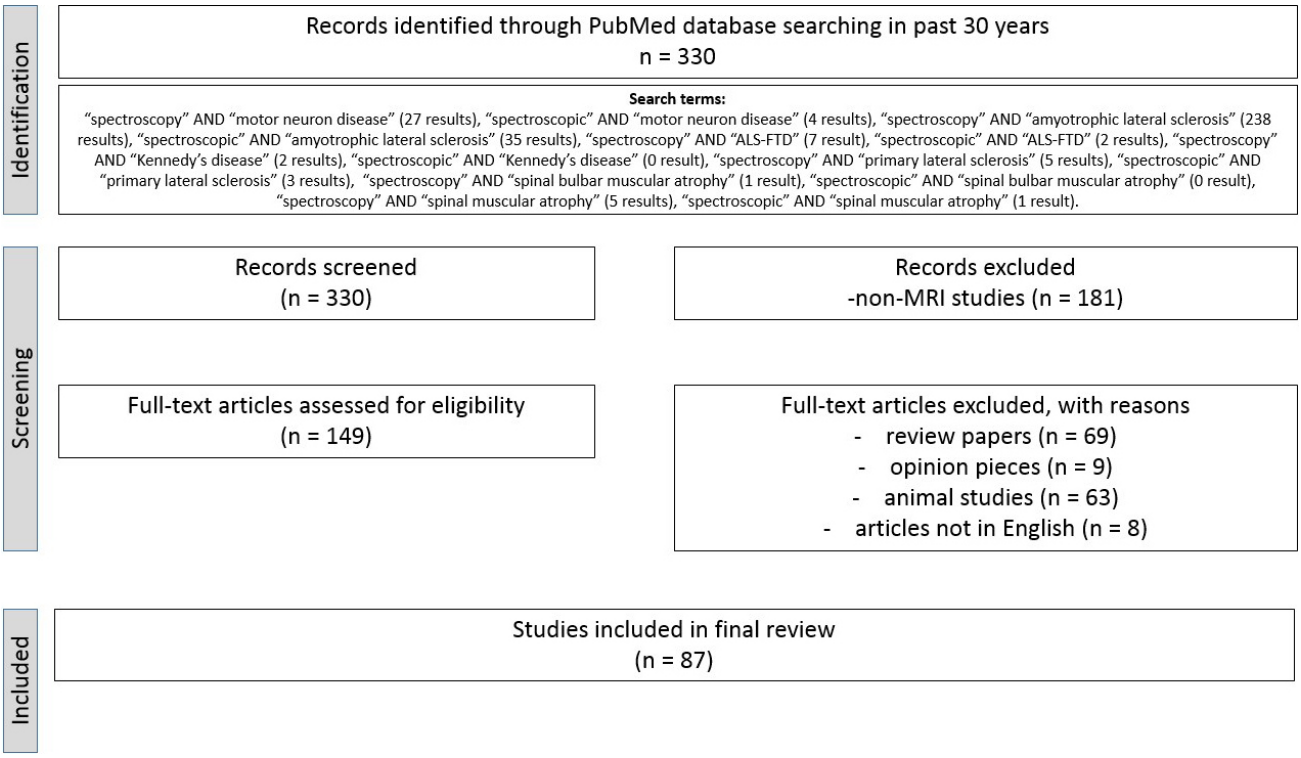

Fig. 2. Systematic review flow chart.

evidence of metabolic changes in prefrontal regions and subcortical structures [27]. However, the profile of neurometabolic changes is relatively under-evaluated in other MND phenotypes, such as primary lateral sclerosis (PLS), progressive muscular atrophy (PMA), spinal bulbar muscular atrophy (SBMA) or Kennedy disease, spinal muscular atrophy (SMA). Moreover, more than 50% of ALS show cognitive impairment [28–31] which is not restricted to traditional frontal-related executive dysfunction [32–34] and corroborates with structural and functional alterations in cross-sectional and longitudinal MRI studies [7,35–43]. In addition, other MND phenotypes present variable changes across different cognitive functions [44,45] in line with neuroimaging findings [46–51]. However, neurometabolic changes in extra-motor regions are only seldom examined and mainly in ALS [27].

Accordingly, the aim of this review is to systematically review the current literature of MR spectroscopy studies in MNDs, highlight clinical implications compared and/or in addition to gold standard techniques, identify pertinent gaps in the literature, evaluate the clinical and academic role of MR Spectroscopy and outline future directions.

2. Methods

A formal literature review was conducted on PubMed between September 2021 and January 2022 in accordance with the PRISMA 2020 guidelines. Two reviewers (F.C. and P.B.) conducted the search and data extraction independently. The search term "spectroscopy" and "spectroscopic" was paired individually with the following keywords: "amyotrophic lateral sclerosis", "motor neuron disease", "PLS", "SBMA", "Kennedy's disease", "PMA", "SMA", "ALS-FTD" (Fig. 2). Selection criteria included original research articles pertaining to spectroscopy brain studies, published during the past three decades. Only human studies were reviewed. Only articles in English were selected for review. Opinion pieces, previous review articles, case reports, research studies without imaging were not reviewed. The reference list of identified papers were also reviewed for articles that might have been missed. We developed an MS EXCEL-type database including specific fields based on which we comprehensively and quantitatively reviewed all studies. The following fields/variables were included for data extraction: first author; year of publication; study sample size; patients' sample size; healthy controls' sample size; patients' and healthy controls' age (mean $\pm$ SD); study design (cross-sectional; longitudinal); type of longitudinal study; MRI field strength; MRS technique; MRS acquisition method (single voxel, multi-voxel, whole-brain); MRS target region; MRS processing software; MRS metrics (absolute values, ratios, institutional units); supporting imaging data; available clinical data (El Escorial diagnostic criteria; ALSFRS/ALSFRS-R; disease duration; progression rate; onset type; treatment with riluzole; respiratory metrics; motor tapping rate; neuropsychological measures); supporting genetic data; supporting post-mortem data; MRS findings; correlations between MRS

findings and clinical data. For each MRS paper, we have examined the results sections and extracted all findings regarding baseline and follow-up group comparisons and positive/negative correlations between MRS findings and available clinical, additional imaging, genetic and/or post-mortem data.

3. Results

Based on the above search strategy, a total of eighty-seven original research studies were identified. Forty-one studies were published after 2010 (**Supplementary Table 1**), thirty-two studies between 2001–2009 (**Supplementary Table 2**) and fourteen studies before 2000 (**Supplementary Table 3**).

3.1 Study Design

The majority of the studies are cross-sectional (62/87 studies) whereas among the 25 longitudinal studies, 10 studies examine pre- and post-treatment metabolic changes, i.e., riluzole [10,52,53], gabapentin [53,54], creatine supplementation [55,56], brain-derived neurotrophic factor (BDNF) [57], olfactory ensheathing cells transplantation [58], minocycline [59], growth hormone [60], and stem cell [61].

3.2 MND Patient Groups

Most of the studies have recruited patients with definite, probable, possible, and clinically probable laboratory supported ALS. The total number of ALS patients included in these studies ranges from n = 1 [62] to n = 154 [63]. Currently, the largest total number of ALS patients in a single-centre study is n = 154 [63] compared to a multicentre study including as many as 74 ALS patients [64]. Only few studies include non-ALS MND groups, i.e., PLS [65–70], PMA [66,69,71–73], SBMA (Kennedy disease) [74–76], presymptomatic SOD1+ individuals [11]. The total number of PLS patients ranges from n = 6 [66,69] to n = 18 [68], the total number of PMA ranges from n = 2 [73] to n = 9 [66,69], the total number of SBMA (Kennedy disease) ranges from n = 5 [74] to n = 10 [76] while the only presymptomatic study includes 24 SOD1+ individuals [11]. Across the 87 studies, 45 studies have a disease sample size between 1–15 patients, 27 studies have a disease sample size between 16–30 patients, nine studies have a disease sample size between 31–50 patients and six studies have a disease sample size larger than 50 patients.

3.3 Reference Groups

In the majority of studies, the ALS patients are contrasted to demographically-matched healthy individuals. Ten studies do not include a healthy control group [10,15,56,59–61]. Six studies include a disease-control group other than MND, i.e., MS, chronic polyneuropathy, and acute polyneuropathy [77], patients not meeting the El Escorial criteria [78], Alzheimer's disease [18], other neurological diseases or various neurological diseases restricted to peripheral nerves [79].

3.4 Demographic and Clinical Profiles

Four studies do not directly report participants' age [15,63,68,80]. Mean patients' age ranges from 45.75 years [81] to 67.7 years [82]. One study includes only male patients [83]. A comprehensive clinical characterisation of study participants' clinical profiles is important to develop meaningful imaging markers. The focus of clinical variables varies among the identified studies. The disease duration profile of patients enrolled in the studies varies significantly ranging considerably even within the same study, e.g., 0–82 months [83] while 17 studies do not directly report patients' disease duration [14,18,53,55,61,63,68,80,84–92]. Age onset is reported in eight studies [15,67,74–76,93–95]. Most studies (57/87) focus on motor-related disease severity, mainly measured using ALSFRS or ALSFRS-R, but five out these 57 studies do not provide any quantitative data for disease severity [11,52,84,89,96]. Onset type (e.g., bulbar and spinal symptoms) is reported in 43 studies [12,15,56,58–61,64,67,70,71,77,79,81,83,84,89,93,94,97–120]. Progression rate is reported in 13 studies [64,65,70,82,94,97,101,103,107,121–124]. Riluzole treatment is reported in 15 studies [15,52,56,59,60,93,99,102,108,117,121,125–128]. Respiratory status (i.e., forced vital capacity, slow vital capacity, non-invasive ventilation) is only available in 19 studies [11,52,54,61,65,66,69,97–99,101,105,106,111,117,122,125,128,129]. Tapping rate is measured in eight studies (only finger [67,90,103,123], both finger and foot [52,65,111,115]), yet in one study data are not reported [52]. Additional cognitive status is only rarely reported. Interestingly, only 11 studies include cognitive scales or tests, i.e., mental status/cognitive screening: Mini-Mental State Examination (MMSE) [62,71,82,111,117], Addenbrooke's Cognitive Examination (ACE) [109], Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [64,93,121]; verbal fluency [71,98,109,120]; executive functions: Wisconsin Card Sorting Test (WCST) [62,117,120]; memory tests: Rey Auditory Verbal Learning Test, Recognition Memory Test, Consonant trigrams test [62,120]; perceptual and constructional dexterities: Motor Free Visual Perception Test [120], Block Design [62,120]; expressive language: Cookie Theft - Boston Diagnostic Aphasia Examination [120]; behavioural inventories [62,120]. In addition, one study includes cognitively preserved patients [70] and another study provides patients' cognitive status (preserved vs impaired) [64] but additional information regarding patients' cognitive evaluation is not provided.

3.5 Target Brain Tissue

Except from the three whole-brain studies which enable the identification of metabolic changes in both motor and extra-motor regions [83,85,107], almost all

single and multi-voxel studies focus on motor-related regions (precentral gyrus, supplementary motor area, premotor cortex, postcentral gyrus, subcortical motor related WM areas (corpus callosum, coronal radiata, internal capsule, cerebral and cerebellar peduncles)). Extra-motor regions are examined in 25 single- or multi-voxel studies [12,52–54,56,62,64,71,73,87–90,98,101,109,111,112,117,120,123,126,129–131]; interestingly, only five of them are dedicated extra-motor studies but mostly examine frontal areas [56,71,98,109]. Basal ganglia and thalamus are examined in one multi-voxel study [111], brainstem in 14 studies [53,59,67,74–76,80,101,102,112,121,127,132,133] and cerebellum in one single-voxel study [72]. On the other hand, there are three studies focusing on the cervical cord [11,106,110].

3.6 Genetic Screening

None of the studies report genetic screening for the GGGGCC hexanucleotide repeat expansions. Two studies use genetic testing for SOD1 [11,72], one study reports genetic screening for six familial ALS included in the study [69] while one study provides data for TSPO Ala147Thr polymorphism based on 11C-PET (11C-PBR28) [101]. Genetic analysis for the number of CAG repeats is finally reported in three studies including patients with SBMA (Kennedy disease) [74–76].

3.7 Supporting Post-Mortem Pathological Validation

Supporting post-mortem pathological validation is provided only for a subgroup of patients in three studies: 6/25 patients [82], 3/43 patients [66] and 11/164 patients [63].

3.8 Supporting Imaging and Neurophysiological Modalities

Twenty-three studies include additional imaging modalities. Specifically, 17 studies use DTI [66,69,77,78,81,82,84,93,94,97,101,107,113,114,124] or DWI [62,70], seven studies provide signal intensity changes [68,78,81,95,112,119,124,130], four studies report GM volume or thickness changes [68,97,119,131], one study uses resting state fMRI [82], one study uses T1ρ [97], one study uses magnetization transfer imaging [70] and one study uses 11C-PET (11C-PBR28) [101]. With regards to additional neurophysiological measures, three studies examine the functional integrity of the corticospinal tract using transcranial magnetic stimulation (TMS) [66,67,92,93].

3.9 Field Strength, Acquisition (Single-Voxel, Multi-Voxel, Whole-Brain) and Post-Processing Techniques

Forty-five studies use 1.5 T (four are published between 2011–2016; [61,84,106]), one study uses 2 T [62], thirty-seven studies use 3 T (among which one is published before 2000; [133]), one study uses 4 T [120], one study uses 3 T and 7 T [102] and two studies use 7 T [121,122]. Forty-nine studies apply a single-voxel technique, 23 studies apply a multi-voxel technique, one study uses both single-voxel and multi-voxel techniques while four studies apply whole-brain spectroscopy. Two studies use diffusion tensor spectroscopy [100] and diffusion weighted spectroscopy [93] while one study uses cranial phosphorus-31 MRS (31P-MRS; [125]) instead of ^{1}H-MRS. With regards to data analysis software, 14 studies do not provide specific details, 24 studies use the LCModel, five studies use the MIDAS, three studies use the jMRUI, one study uses the TARQUIN, and 38 studies use either in-house or manufacturer-provided software. Almost half of the studies do not apply tissue segmentation and correction for GM, WM, and CSF within each VOI. With regards to the reporting metrics, 52 studies provide only ratios, 10 studies provide only absolute values, 24 studies provide absolute values and ratios, and one study reports institutional units and ratios.

3.10 Neurometabolic Changes in ALS

3.10.1 Cross-Sectional Studies

3.10.1.1 Cortical Motor Regions. MR Spectroscopy studies verify motor abnormalities in ALS based on the pattern of metabolic changes in primary motor cortex/precentral gyrus and other motor regions, including the supplementary motor area (SMA). Several studies provide evidence of reduced NAA [66,72,88,99,119,122,127], tNAA [122], NAA/Cr [63,68,91,94–96,99,103,112,113,115–117], NAA/tCr [66,105], NAA/Cho [91,92,95,99,113,115,116,123,131], NAA/Glx [99], NAA/Cho+Cr [90,95], NAA/mIns [99,102,115], tNAA/Cr [126], tNAA/mIns [102]. Interestingly, abnormal NAA/Cr ratio is found in most patients in the largest single-centre study which also reports lower NAA/Cr ratio in patients with definite UMN signs compared to patients with no UMN signs [63]. Precentral gyrus reduced NAA/Cr and NAA/Cho is found both in affected and non-affected hemispheres of ALS patients when compared to healthy individuals while NAA/Cho is significantly lower in the affected compared to non-affected hemisphere within ALS [114]. Other relatively common metabolic changes in primary motor cortex include increased mIns [99,127], mIns/NAA [99], mIns/Cr [99,115] and mIns/tCr [102]. Increased mIns/Cr ratio is reported to ALS patients with UMN involvement compared to patients with LMN involvement [77]. Lower NAA/Cr ratio is associated with lower FA and reduced 11C-PBR28 in bilateral precentral gyri while higher mIns/Cr is associated with lower FA and reduced 11C-PBR28 in bilateral precentral gyri [101]. Two whole-brain studies identify reduced NAA/Cr in right precentral gyrus [85] and simultaneous decreased NAA and increased Cho in motor and premotor areas extending along motor fibers based on multivariate but not univariate analysis [83]. Reduced GABA is also demonstrated in two studies [108,127].

The NAA/Cho ratio in primary motor cortex is found to have the highest sensitivity and specificity compared

to other ratios (NAA/Cr and Cho/Cr) in discriminating ALS from healthy individuals [116]. The combination of NAA/Cho+Cr ratio and hypointensities in precentral gyrus and hyperintensities in the corticospinal tract have a diagnostic accuracy of 79.4% compared to the diagnostic accuracy of 72.3% for the precentral gyrus NAA/Cho+Cr alone [95]. By comparing the MR Spectroscopy changes with classic MRI metrics, Chan and colleagues (1999) report a sensitivity of 73% for motor cortex NAA/Cr (vs 38% for corticospinal tract hyperintensities and 23% for widening of central sulcus) in ALS, 88% (vs 12% for corticospinal tract hyperintensities and 12% for widening of central sulcus) in ALS with probable UMN signs, 67% (vs 10% for corticospinal tract hyperintensities and 14% for widening of central sulcus) in PLS and 17% (vs 0% for corticospinal tract hyperintensities and 38% for widening of central sulcus) in non-UMN disorders [68].

Two multimodal studies provide interesting findings with regards to the pattern of motor metabolic changes in different El Escorial groups (i.e., definite, probable, possible, suspected). The NAA/Cho in primary motor cortex shows variable percentage of changes across El Escorial groups and compared to TMS, MR Spectroscopy detects more patients with UMN involvement in the suspected group while the TMS detects more patients with UMN involvement in possible, probable and definite El Escorial groups [92]. According to a more recent single-voxel MR Spectroscopy and DTI study, it appears that increased mIns/Cho and Cho/Cr ratios in precentral regions are found in all ALS subgroups (definite/probable, possible/suspected, suspected) compared to healthy individuals while reduced NAA/Cr is found only in definite/probable ALS patients [124].

One of the most recent studies included in this review combines MR Spectroscopy, DTI and resting-state fMRI to examine SMA abnormalities in 25 ALS patients; patients show increased Glu/NAA and Glu/GABA compared to healthy individuals but no differences in absolute values (Glu, NAA, GABA), fractional anisotropy (FA) or regional homogeneity (ReHo) of the SMA [82].

3.10.1.2 Cortical Extra-Motor Regions. Most studies focus and/or report changes in NAA absolute values or ratios relative to healthy individuals. Except for three studies that do not identify any frontal-related metabolic changes [87,88,90], changes are evident in medial prefrontal cortex (reduced NAA/Cho: [64]; reduced NAA/mIns: [109]), mid-cingulate cortex (reduced NAA/Cho: [123]) and posterior superior frontal gyrus (reduced NAA/Cr: [73]). In a longitudinal study where bulbar-onset and limb-onset ALS patients were compared to healthy individuals, baseline reduced NAA/Cr is reported in ACC in bulbar-onset ALS but not in limb-onset ALS but six months after the first MRI scan, reduced NAA/Cr is also found in limb-onset ALS patients [120]. Atassi *et al.* [56] and colleagues examine the metabolic profile after different dosages of creatine supplementation and report increased Cr and Cr/Cho and decreased Glx in the frontal cortex between baseline (pre-treatment) and the higher dosage of creatine. Parietal regions are seldom examined and most cross-sectional [89,90,117] and longitudinal [52,54,87,129] studies fail to identify any changes, with the only exception been the pioneer MR Spectroscopy study by Pioro *et al.* [73] and colleagues where decreased NAA/Cr is found in superior parietal gyrus/precuneus, although to a lesser extent than in primary motor cortex. On the other hand, even though several single or multi-voxel MR spectroscopy studies often include occipital regions as a reference region and do not find significant metabolic changes [53,71,88,89,112,130,131], a whole-brain cross-sectional study in 16 ALS patients and 10 healthy individuals demonstrates reduced NAA/Cr and NAA/Cho not only in inferior frontal operculum, caudate, Hesch gyrus, and supramarginal gyrus but also in several occipital regions (lingual gyrus, superior occipital lobe, inferior occipital lobe, cuneus) [85]. Interestingly, none of the available studies examine metabolic changes in temporal regions.

3.10.1.3 Subcortical Structures. There are only few available but inconsistent data for the metabolic profile of subcortical structures [85,111,123]. A 3 T study dedicated to basal ganglia and thalamus demonstrates reduced NAA and NAA/Cho and increased Cho in lentiform nucleus and thalamus but not in the caudate in ALS compared to HC [111]; however, a 1.5 T study failed to reproduce these findings in the thalamus [123]. Another whole-brain study provides evidence of reduced NAA/Cho, NAA/Cr in left caudate but does not identify any metabolic changes in the thalamus [85].

3.10.1.4 Brainstem. Most studies indicate reduced NAA or NAA/Cr ratios in brainstem [53,102,127,132,133]. Other studies failed to identify metabolic changes in the NAA ratios (NAA/Cr, NAA/Cho) of the brainstem [112]. Increased Glx/PCr ratio is reported in one study [132], yet some others failed to identify changes in Glu or Glx [53]. Of note, findings from a recent longitudinal study indicate increased Glx [121].

3.10.1.5 Cerebellum. Only one study specifically focuses on the cerebellum [72]; however, no significant differences were identified in Cho, Cr/PCr and NAA.

3.10.1.6 White Matter. Among the studies which examine metabolic changes in white matter, most of them focus in the motor WM pathways and the corticospinal tract [15,58,62,67,70,81,83,84,86–88,90,96,107,108,113,127,128], two studies examine the corpus callosum [84,93] and one study examines frontal WM [62]. One of the most consistent finding is reduced NAA and/or NAA/Cr in subcortical mo-

tor WM [127], posterior limbs of the internal capsule [96], corona radiata [113], centrum semiovalis [70], cerebral peduncle and internal capsule [62], and CST [107,128]. Some studies that examine metabolic changes throughout the entire CST [128] or combined parts of it [81,90] and different segments find reduced NAA throughout the course of the CST [128] or the combined segments [81] but not on every CST segment [90,128]. Significant metabolic alterations have also been reported in motor and premotor areas extending along motor fibers (decreased NAA and increased Cho) using unbiased whole-brain MR spectroscopy [83]. Reduced NAA/Cr and NAA/Cho in CST appear to be found not only in definite and probable but also in possible ALS relative to healthy individuals [84]. Interestingly, a multimodal MR Spectroscopy and DTI study indicates reduced NAA throughout the course of the CST and reduced fractional anisotropy (FA) and increased mean diffusivity (MD) but highlights the greater anatomical specificity compared to FA and MD [107]. Other metabolic changes include higher mIns in subcortical motor WM [127], increased Cho in the entire CST, increased Cho/NAA in the entire CST and different segments [128], increased Glx/Cr and Glu/Cr [96] and Cho [88] in posterior limbs of internal capsule, reduced Glx/Cr in cerebral peduncle and internal capsule and reduced Cho/Cr in internal capsule [62]. Absence of metabolic changes including NAA are more rarely reported [88,108]. On the other hand, reduced NAA/Cr and NAA/Cho in definite and probable ALS but not in possible compared to healthy individuals has been reported in the splenium of the corpus callosum [84]. A more recent study does not reproduce these findings in the corpus callosum using DTI-MRS but identifies higher D(tNAA) variability in ALS [93].

3.10.1.7 Spinal Cord. The few MR Spectroscopy studies indicate reduced metabolic ratios in ALS compared to healthy individuals: NAA/Cr [11,106,110], NAA/Cho [11], NAA/mIns [11,106,110], mIns/Cr [11,106] and Cho/Cr [110].

3.10.2 Longitudinal Studies

Among the 25 longitudinal studies, 14 studies examine neurometabolic changes in ALS over varying time intervals [15,18,65,69,78,86,87,100,102,118,120,121,130,134] and all but five [64,86,87,120,130] focus exclusively on motor regions. Earlier studies provide evidence of progressive decline in NAA [130] and NAA/Cho [118,134] with the highest changes being observed in patients with normal baseline spectroscopy data [14]. In a longitudinal study (0 m, 3 m, 6 m) of metabolic changes in GM motor region and subcortical paraventricular WM including the corticospinal tract, there is a progressive reduction of the NAA (0 m vs. 3 m) for the clinically less affected hemisphere and of NAA (0 m vs. 6 m) for both the less and more affected hemisphere with regards to the GM motor region but not the WM [15]. In a recent study where ALS patients are subdivided into fast and slow "progressors" and those with UMN and LMN involvement, it appears that progressive decline in NAA/Cr and NAA/Cho is preferentially detected in "fast progressors" as well as patients with predominant UMN involvement [64]. A previous comparative study of ALS and AD where motor cortex and mesial temporal lobe is examined in each group, respectively, finds a general trend for further reduction in motor cortex NAA/Cho over time but inter-subject variability in ALS and a trend towards progressive reduction of mesial temporal lobe NAA/tCr and NAA/Cho in AD [18]. However, the study does not examine mesial temporal lobe longitudinal changes in ALS. Few studies have examined longitudinal alterations in association with varying levels of diagnostic certainty (definite, probable, possible, suspected ALS) but yield inconsistent findings [78,86,87]. Interestingly, among the studies which examine both motor and extra-motor longitudinal changes in ALS, there are inconsistent findings with some studies reporting no prefrontal changes [64], other studies showing reduced in NAA at 9 m but not earlier at 3 m in probable/definite but not in possible/suspected ALS [86] and some other studies identifying reduced NAA/Cr both in motor cortex and anterior cingulate cortex in limb- but not in bulbar-onset ALS [120].

In addition, there are 11 studies which evaluate specific treatment-related neurometabolic changes [10,52–56,58–61,129]. In treatment studies of riluzole, there is evidence of increased NAA/Cr ratio in motor cortex [10,52] and SMA [52]. Two studies examine the neurometabolic changes after creatine supplementation in motor cortex [55] and frontal cortex [56]. They show post-creatine supplementation decline in NAA/tCr, NAA/Cho, Glx/tCr and Glx/Cho ratios in healthy individuals and decline in Glx/tCr and Glx/Cho but no changes in NAA/tCr (which was reduced at baseline relative to healthy individuals) and NAA/Cho ratios in ALS [55]. It also appears that there is a significant Cr increment, Glx decline and Cr/Cho increment in the frontal cortex between the pre-treatment and the highest dosage of creatine supplementation [56]. Longitudinal studies examining the effect of stem-cells [61], growth-hormone [60], minocycline [59], gabapentin [54] do not find any significant post-treatment differences compared to the baseline metabolic profile of ALS.

3.11 Neurometabolic Changes in PMA

There are only few MR Spectroscopy studies that have recruited patients with PMA. Among the five of them which are included in the present review [66,69,71–73], only four provide a direct comparison between PMA and HC [66,72,73]. Two earlier studies do not find any metabolic changes neither in the motor cortex [72,73] nor in sensory and parietal regions [73] or the cerebellum [72]; however, more recent studies report decreased NAA/tCr ratio but not NAA in PMA compared to HC [66,69]. In another study

where metabolic changes are examined in dorsolateral prefrontal cortex (DLPFC), a supplementary analysis in a subgroup of patients with PMA shows higher NAA/Cr in left DLPFC and average DLPFC compared to a group of definite, probable and possible ALS [71].

3.12 Neurometabolic Changes in PLS

All available data on PLS are related to motor regions. There is consistent evidence for reduced NAA/Cr ratios in PLS compared to healthy individuals [65–69] and to advanced-phase ALS [70]. One study also reports increased mIns compared to healthy individuals [65].

3.13 Neurometabolic Changes in SBMA (Kennedy Disease)

The few MRS studies in SBMA (Kennedy disease) indicate inconsistent findings [74–76]. Two long echo-time MR spectroscopy studies demonstrate reduced NAA/Cr in motor cortex [74,76] and NAA/Cho in motor cortex and brainstem [76]. A short echo-time study fails to reproduce these findings in motor cortex where only increased mIns was found [75].

3.14 Neurometabolic Changes in Presymptomatic Cases

Reduced mIns/Cr, NAA/Cr and NAA/mIns in the cervical cord (C1-C2) is reported in the only study including presymptomatic cases compared to healthy individuals [11].

3.15 Post-Mortem Correlates

Even though there are few studies with available post-mortem data, none of them provide any direct correlation with MRS findings.

3.16 Clinical Correlates

3.16.1 Disease Duration

Correlations between disease duration and metabolic ratios in motor-related GM and WM regions are found in number [103,104,112,113,118,127] but not all [10,52,70,74,76,77,94,95,105,107,115,116,124] studies. Moreover, there are conflicting findings, i.e., positive association with motor cortex NAA/Cr [103] and negative association with right precentral gyrus NAA/Cr [112], positive association with precentral NAA/Cho [113] and negative association with primary motor cortex [112,118] and medulla oblongata [112] NAA/Cho. Lower NAA/Glu [104] and GABA [127] in motor cortex and higher mIns in subcortical WM caudal to the motor cortex [127] are also found to be associated with longer disease duration.

3.16.2 Disease Severity and Progression Rate

Studies that examine the association between metabolic changes in MND and disease severity (mostly measured through ALSFRS or ALSFRS-R) or progression rate provide inconsistent findings (**Supplementary Table 1**) and there are several studies that fail to establish any relation [10,52,71,76,77,94–96,103,105,110,113,115,116].

Positive correlations between disease severity and metabolic profile of motor regions (GM and/or WM) is found in several studies (NAA: [66,69,107,112,127]; NAAx: [78]; tNAA/Cr: [102,126]; NAA/Cr: [99,101,114]; NAA/Cho (caudate): [111]; NAA/Glx: [99]; NAA/mIns: [99]; tNAA/tmIns: [102]; Glu [102]; mIns: [78,102]; NAA/Cr+PCr: [12]; Cho/Cr+PCr: [12]. On the other hand, negative association are also reported with motor regions mIns [78,99], Glx [99], mIns/Cr [101], NAA/Cho [112], Glx/PCr [120]. In addition, a negative association is reported for brainstem NAA/Cr ratio and the severity of brainstem signs and symptoms [133]. The only 31P-MRS study in ALS also reports a negative association between brainstem phosphocreatine and ADP and disease severity measured through ALSFRS-R [125]. Higher NAA/Cr and NAA/mIns in the cervical cord of ALS patients is associated with higher ALSFRS-R and non-bulbar ALSFRS-R and lower annual decline of ALSFRS-R and non-bulbar ALSFRS-R [106]. In a longitudinal study where olfactory ensheathing cell transplantation was applied in seven patients who underwent MR Spectroscopic examination focused on motor regions (cerebral peduncle, genu and posterior limbs of internal capsule, corona radiata, precentral gyrus), higher NAA/Cr was found in certain anatomical positions which confirm the neurological and EMG findings in two cases with ALSFRS improvement following the transplantation [58]. In another longitudinal study with stem-cell transplantation, baseline (M0) NAA/Cr ratios in the precentral gyrus at the dominant and non-dominant hemispheres are found to be associated with follow-up (M12) ALSFRS-R [61].

Only few studies establish an association between disease progression rate and metabolic changes. Disease progression rate is negatively associated with motor cortex NAA [89,97], tNAA/tCr [122] and NAA/Cho [113,123]. In a recent longitudinal study where ALS patients are categorized as fast and slow progressors, motor cortex NAA/Cr ratio is lower in fast compared to slow progressors patients at baseline and further decline in motor cortex NAA/Cr and NAA/Cho ratios is reported in fast progressors ALS [64].

3.16.3 Onset Type Phenotypes

Few studies examine differences in metabolic profile between different onset-type phenotypes. Bulbar-onset ALS patients show reduced motor cortex NAA [99,119] and NAA/Cr+PCr [12] compared to limb-onset patients in another diffusion tensor spectroscopy study, limb-onset patients show a more widespread pattern of alterations characterized by increased MD tCr and MD tCho, AD tCho, RD tCr and RD tCho, and decreased tNAA/tCr and tNAA/tCho in precentral gyrus compared to healthy individuals while bulbar-onset patients show only increased MD tNAA and tNAA/tCho [100]. However, there are studies that fail

to establish any association between onset type and a different pattern of metabolic alterations in motor regions [52,108,118] or dorsolateral prefrontal cortex [71].

3.16.4 Motor Dexterity (Finger Tapping, Foot Tapping)

Finger and foot tapping rate are used as simple but objective measurements of UMN function in MND and other clinical groups with UMN deficits that show reduced rate to produce rapid and repetitive movements of the hands and feet. Some studies report positive correlations between finger tapping rate and motor cortex NAA [66,69], NAAx [78] and NAA/Cr [67,103], as well as between foot tapping rate and NAA/tCr [69]. Negative correlations are noted between finger tapping rate and NAA/Cho in mid-cingulate cortex [123], Cho/NAA in the precentral gyrus part of the corticospinal tract and centrum semiovalis [128] and mIns in motor cortex [78], as well as between foot tapping rate and Cho/NAA in posterior limbs of the internal capsule [128]. Other studies do not find any association [52].

3.16.5 Survival

Only few reports are available regarding the predictive role of metabolic changes in motor and extra-motor regions and patients' survival. Primary motor cortex NAA/Cho ratio, older age and shorter symptom duration are found to be associated with reduced survival in a previous ALS study [116]. In a more recent study, NAA/mIns in the medial prefrontal cortex is reported as an independent predictor of survical in ALS patients two but not five years after the MRI scan in addition to age, ALSFRS-R and forced vital capacity while the same metabolic ratio is also identified as the only significant predictor of survival in a multivariate Cox regression model [98].

3.17 Cognitive Correlates

As cognitive measures are only seldom used and extra-motor regions are rarely examined in the MR Spectroscopy studies, there are only scarce reports of the association between cognitive processes and metabolic changes in MND [64,71,109,117]. Among the available MR Spectroscopy studies with cognitive measures, some studies report a positive association between NAA/Cr in frontal/prefrontal areas and performance in executive measures [71,117] and identify reduced NAA/Cho in cognitively-impaired ALS compared to HC but not compared to cognitively-unimpaired ALS [64]. Patients with ALS and impaired letter fluency show reduced left and average dorsolateral prefrontal cortex NAA/Cr compared to ALS patients with normal letter fluency, even after excluding a subgroup of PMA [71]. There is no reported correlation between non-executive measures (i.e., language, memory, perception, constructive dexterities) and metabolic changes in MND groups.

4. Discussion

While structural, diffusivity and functional methods are often applied in conjunction in MND [135], spectroscopy is often performed in isolation. Only a minority of the neuroimaging community in MND would routinely use MRS techniques, which has led to strikingly divergent research strategies in structural and spectroscopic imaging. While MRS studies pioneered innovative designs, such as monitoring drug response [10,52,54,129], evaluation of cord metabolites [110], assessment of changes in non-ALS MNDs [74–76], structural studies turned their attention to frontotemporal regions [136], infratentorial changes [137], extra-pyramidal deficits [138], and machine-learning applications [139,140] relatively earlier. The relevance therefore of reviewing advances in MRS in MND points beyond metabolic imaging, as it offers valuable lessons for non-spectroscopic imaging in MND.

4.1 Methodological Considerations

The review of published MRS studies reveals diverse methodological approaches. The majority of MRS studies adopt a single-voxel approach and only few capitalise on recent advances in whole-brain spectroscopy [85,107]). With the exception of a 4 Tesla [120] and three 7 Tesla study [102,121,122], most studies relied on 1.5 or 3.0 Tesla field strength. The vast majority of studies applied 1H-MRS and only few utilised MRSI [10,52,54,60,66,67,70,71,73,83,84,86–88,90,95,97,103,113,114,116,123], diffusion-tensor spectroscopy [93,100], or 31P-MRS [125]. With the exception of two superior spinal cord studies [11,106,110] all studies focused on the evaluation of cerebral metabolites. The most commonly evaluated brain region was the motor cortex, but targeted brainstem [80,101,102,121,132,133] and innovative thalamus studies were also identified [13,123]. Strikingly few studies have evaluated frontotemporal regions [71,86], and only few studies acquired spectra from parietal or occipital regions [12,89,90,126,131] often as 'reference voxels'. A potential limitation of our study is that search and data extraction was conducted on a single database. Methodological considerations have also been comprehensive reviewed in other clinical groups [141].

4.2 Clinical Associations

The majority of MRS studies in ALS report composite functional disability scores (ALSFRS) and some pursue direct correlation analyses between cerebral metabolite ratios and ALSFRS even though ALSFRS is also affected by anterior horn degeneration. To delineate the clinical impact of UMN degeneration some studies record finger tapping rates [103,123], foot tapping [52,142], 'rapid bulbar muscle movements' [13], UMN scores [77], which allow for more accurate correlation analyses. Interestingly, the presence of pseudobulbar affect (PBA) or a positive jaw jerk is not commonly reported despite their association with corticob-

ulbar dysfunction [143,144]. Site of onset (bulbar/spinal) is often reported albeit the relevance of onset may be relatively limited by the time spectra are acquired [145]. While site of onset is invariably reported, side of onset (left or right) is seldom noted, despite its relevance to the laterality and symmetry of findings. Progression rates, clinical staging, respiratory function profiles, Riluzole therapy are commonly reported among core clinical variables even if clinico-metabolic associations are not sought. In striking contrast to structural studies, accompanying cognitive and behavioural information is scarce in published MRS reports. ECAS [64,93,121], letter fluency [71,98,109] or MMSE [71,82,117] is sometimes reported, but detailed neuropsychological testing is seldom available [62,120]. Despite their clinical relevance [146,147] accompanying behavioural data [62], tests of social cognition and apathy are seldom appraised in MRS studiers despite their association with specific brain regions.

4.3 Stereotyped Shortcomings

The review of published MRS studies also reveals common study limitations. Most of the published MRS studies in ALS, particularly earlier studies in the field, only report MRS alterations and corresponding structural, diffusivity and functional changes are not evaluated. More recent studies commonly describe associations with diffusivity changes, typically with fractional anisotropy (FA) [77,93,94,107]. Some studies acquire complementary functional data, such as rsfMRI [82], PET [101] or TMS [66,67,92,93] correlates. The lack of accompanying post mortem assessments makes the histological interpretation of metabolic changes challenging. Only three studies [63, 66,82] described pathological correlates of ante mortem MRS findings. One of the most notable limitations identified is the lack of MRS studies in GGGGCC hexanucleotide repeat carriers in *C9orf72*. While the structural correlates of *C9orf72* have been extensively investigated [137,148,149] the metabolic correlates of this genotype remains to be characterised. With the exception of a few SOD1 studies [11,72], and a few studies in genetically-confirmed SBMA (Kennedy's disease) [74–76] the vast majority of existing MRS studies did not stratify their cohort by genetic profile. This is a missed opportunity at a time when genotype-associated clinical traits, differences in survival, disability profiles are well characterised, and emerging therapies such as antisense oligonucleotides (ASO) target specific genotypes. A notable shortcoming of existing studies is the overwhelming focus on ALS. MNDs encompass a multitude of clinically diverse phenotypes with differences in progression rates [24], survival [150], non-neurological involvement [151], spinal cord pathology [152]. The vast majority of existing MRS studies only evaluate patients with ALS, and only a few investigated changes in PLS [65,66,68], PMA [66,69,72], or SBMA [74–76]. PLS is associated with longer survival than ALS [153] and is a difficult to diagnosis to establish early in the course of the disease [154] as UMN-predominant ALS may present similarly despite running a more sinister course. There is a lack of spectroscopy studies in rare MNDs such as post-polio syndrome (PPS) where progressive changes are sometimes detected decades after the initial cord insult [23]. The characterisation of distinguishing metabolic signatures in non-ALS MNDs should therefore be an important priority of future research. Less than a third of published MRS studies are longitudinal, and the majority of these studies have a mere two-time point design which preclude the assessment of non-linear changes, ceiling and flooring effects. MNDs, ALS in particular, are rapidly and inexorably progressive neurodegenerative conditions, where cross-sectional studies merely offer a snapshot of metabolic change and patients are often in a different stage of their disease trajectory [155]. In cross-sectional studies, the "disease-duration" or "symptom duration" profile of participants is often not reported despite being a key determinant of the detection and magnitude of pathological change. When this information is reported, it is often apparent that patients with a considerable symptom duration range have been included which makes the interpretation of findings difficult. Reporting disease duration is indispensable, as for example the absence of MRS findings may be due to early-stage disease or technical factors. The admixing of patients with very different disease duration may be a necessity due to cohort size limitations, but the impact of disease duration should always be carefully considered. A requisite of future MRS studies therefore is a prospective, multi-timepoint longitudinal design, with a relatively homogenous symptom duration profile at enrolment and uniform follow-up intervals. While presymptomatic structural and diffusivity changes have been consistently described in ALS [156,157], there is also a striking scarcity of metabolic studies of asymptomatic mutation carriers [11]. It is currently unclear if early metabolic changes may precede structural degeneration and augur focal vulnerability before structural neurodegeneration ensues. If that is the case, MRS may be particularly useful to detect early pathophysiological changes, which may have ramification for recruitment in pharmacological trials. The nuanced characterisation of metabolic alterations in asymptomatic mutation carriers may help to elucidate anatomical propagation patterns [158] and may have a prognostic role in predicting age of symptom onset. The challenges of recruiting asymptomatic mutations carriers are well known, but viable strategies have been developed to acquire large multi-site datasets and meaningfully interpret premanifest radiological changes [156,159,160].

4.4 Methodological Trends in MR Spectroscopy

On the review of studies by date of publication, cohesive methodological trends emerge: (1) with ever improving scanner designs, studies increasingly rely on higher field-strengths, (2) with the recognition of extra-motor

manifestation in ALS, studies increasing evaluate non-motor regions, (3) with the availability of whole-brain techniques multiple brain regions are now often simultaneously evaluated [85,107], (4) with the availability of novel pulse sequences, MRS data is increasingly complemented by simultaneously acquired structural, functional or PET data which aid the interpretation of metabolic changes (5) with the availability of open-source software there is a shift from reliance on in-house pipelines to validated robust analysis suites which helps the comparison and replication of research findings.

4.5 Academic Utility

Existing MRS studies in MND indicate that MRS has the potential to define disease associated (PLS, SBMA, ALS) metabolic signatures and capture progressive changes over time. Even though the majority of ALS are sporadic cases with no clear genetic linkage, there is a considerable 10% of cases with familial inheritance [161] and there are collective efforts to study presymptomatic ALS cases and identify biomarkers [162–164]. With the advent of whole-brain protocols MRS could be utilised to clarify anatomical propagation patterns from the presymptomatic phase, through the peri-diagnostic stage, well into the late phases of the disease and help to establish if metabolic alterations precede, coincide or follow structural and diffusivity changes. Multi-time point metabolic profiles may meaningfully contribute to the corticofugal versus corticopetal debate, and offer validation opportunities for proposed histopathological staging systems *in vivo* [165,166]. Animal imaging studies in MND rarely implement MRS despite the high-field strength generated by small-bore scanners and the opportunity to link specific spectral profiles to histopathological changes.

4.6 Potential Clinical Utility

The insights gained from MRS studies in MND to date have not been translated into practical clinical applications. Given the differences of spectra between patients and controls, the early diagnostic utility of spectroscopy in suspected MND cases and the potential role in distinguishing MND phenotypes have not been fully explored. ROC curves and various machine-learning frameworks [167] in structural imaging suggest that imaging may have a role in delineating MND phenotypes [47] or distinguish early-stage ALS patients from healthy individuals [168] with adequate accuracy. The clinical utility of MRS should not only be examined form a diagnostic standpoint, but its potential to track longitudinal changes and be used as a putative monitoring marker in clinical trials should also be explored. Finally, similar to structural imaging [169,170], the prognostic correlates of metabolic alterations should be carefully evaluated.

4.7 Future Directions

MRS needs to be integrated into multimodal imaging protocols to establish its comparative detection sensitivity, relative advantages and its diagnostic, monitoring, and predictive potential. Future studies should address whether MRS can be developed into a viable clinical tool to differentiate various phenotypes, monitor progression, and detect response to therapy. The practical demands of clinical imaging require relatively short acquisition times, transparent and reliable data interpretation pipelines, uniformity of voxel placement and ease of harmonising protocols across clinical sites. There is a relative urgency to shift the emphasis from descriptive, MRS-only academic studies to the development of protocols with practical clinical utility.

5. Conclusions

MRS remains one of the most promising MR-based imaging modalities in MND, which offers invaluable metabolic data non-invasively. Existing studies have already cemented the academic role of MRS by characterising neurodegenerative processes in MND. An urgent priority of future studies is to define, gauge, and establish the clinical role MRS in expediting the diagnosis, monitoring disease progression and assessing response to therapy.

Author Contributions

The manuscript was drafted by FC, EK, GDA, IS, and PB. EK, GDA and IS contributed to the MRS analyses and the results presented in Fig. 1. The final draft was reviewed for intellectual content by GV, VZ, AM, IE, and MR.

Ethics Approval and Consent to Participate

The acquired spectra presented in Fig. 1 was obtained as part of a research MRS study which was approved by the local ethical committee of Aeginition Hospital; National and Kapodistrian Univeristy of Athens; GR (ΑΔΑ, ΨΔ4846Ψ8 Ν2 – Γ9Φ/06-11-2020); all participants provided informed consent before MRI scanning.

Acknowledgment

We would like to thank the participants of the research MRS study based on which we generated Fig. 1.

Funding

Foteini Christidi is supported by the EU-IKY Scholarship Program (European Social Fund-ESF), the Greek "Reinforcement of Postdoctoral Researchers" grant (5033021) of the "Human Resources Development Program, Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF 2014-2020). Professor Peter Bede is supported by the Health Research Board (HRB EIA-2017-019 & JPND-Cofund-2-2019-1), the Irish Institute of Clinical Neuroscience (IICN), the Spastic Paraplegia Foundation (SPF), the EU Joint Programme – Neurodegenera-

tive Disease Research (JPND), the Andrew Lydon scholarship, and the Iris O'Brien Foundation.

Conflict of Interest

The authors have no conflicts of interest to declare. FC and EK are serving as one of the Guest Editors of this journal. We declare that FC and EK had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to LP.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2103087.

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