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Motor and extra-motor involvement in amyotrophic lateral sclerosis

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SUMMARY

Amyotrophic Lateral Sclerosis (ALS) is primarily associated with motor cortex and corticospinal tract pathology. Based on clinical observations, it is increasingly recognised that extra-motor symptoms add to the clinical heterogeneity of the disease and varying degrees of neuropsychological, extra-pyramidal, and cerebellar involvement are also often observed.

The objective of this work is the radiological characterisation of motor and extra-motor involvement in ALS using multimodal quantitative neuroimaging.

Eighty-five patients and 76 healthy controls were enrolled in this translational research study which has been specifically approved by the appropriate Ethic Committee. All patients underwent standardised clinical assessments and participated in a purpose-designed 3 Tesla magnetic resonance imaging protocol.

Whole-brain and region of interest grey and white matter analyses were performed assessing regional brain volumes and indices of white matter integrity.

Our results suggest that the unifying disease-specific imaging signature of ALS includes precentral gyrus atrophy, corpus callosum degeneration and corticospinal tract involvement. Additionally, considerable extra-motor changes including frontotemporal degeneration have also been identified.

The characterisation of extra-motor motor involvement in ALS has implications for individualised patient care, clinical trials designs, multidisciplinary interventions and the support of family members and caregivers.

PUBLICATIONS AND PRESENTATIONS IN ASSOCIATION WITH THIS WORK

Chipika HR, Finegan E, Li Hi Shing S, Hardiman O, Bede P. Tracking a fast-moving disease: Longitudinal markers, monitoring, and clinical trial endpoints in ALS– accepted for publication on 19 February 2019 Frontiers in Neurology

Bede P, Omer T, Finegan E, **Chipika HR**, Iyer PM, Doherty MA, Vajda A, Pender N, McLaughlin RL, Hutchinson S, Hardiman O. Connectivity-based characterisation of subcortical grey matter pathology in frontotemporal dementia and ALS: a multimodal neuroimaging study. Published Brain Imaging Behav. 2018 Feb 8. doi: 10.1007/s11682-018-9837-9. [Epub ahead of print] PMID: 29423814

Bede P, Finegan E, **Chipika RH**, Li Hi Shing S, Lambe J, Meaney J, Redmond J. Occulomotor Neural Integrator Dysfunction in Multiple Sclerosis: Insights From Neuroimaging. Published Front Neurol. 2018 Aug 23;9:691. doi: 10.3389/fneur.2018.00691. eCollection 2018. PMID: 30190700

Chipika HR. Neuroimaging in ALS. Platform – The Andrew Lydon Award Scholarship Event, Trinity College Dublin, 21 September 2018

Chipika HR. Posterior pathology in ALS: the spectrum of occipital changes, Platform Presentation, Neuroimaging Society of ALS, NISALS, Edinburgh, UK, 5-6 December 2018

Chipika HR, Finegan E, Hardiman O, Bede P. Characterising occipital lobe pathology in ALS using individual patient analyses: the quest for a "posterior" phenotype. Poster presentation International ALS/MND Symposium 2018, Glasgow, UK, 7-9 December 2018

Chipika HR, Finegan E, Iyer P, Omer T,. Doherty M, Vajda A, Pender N, L. McLaughlin RL, Hutchinson S, Hardiman O, Bede P. Distinctive subcortical grey matter signatures along the ALS-FTD spectrum: a multimodal neuroimaging study. Poster Presentation European Network for the Cure of ALS (ENCALS) meeting, Oxford, UK, 20-22 June 2018

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GLOSSARY

2D-DIGE- two-dimensional fluorescence difference gel electrophoresis, **ABG**- arterial blood gas, **ACE-3**- Addenbrookes Cognitive Examination - Third Edition, **ACE-R**- Addenbrookes Cognitive Examination, **AD**- axial diffusivity, **ADM**- abductor digiti minimi, **ADQ**- abductor digiti quinti, **AGA**- arterial gas analyses, **AHB**- abductor halluces brevis, **ALS** – amyotrophic lateral sclerosis, **ALSAQ-40** -ALS assessment questionnaire, **ALS-CBS**- ALS Cognitive Behaviour Screen, **ALS-CFB**- ALS computerised frontal battery, **ALS-FBI**- ALS-Frontal Behavioral Inventory, **ALSFRS-r** - revised ALS functional rating scale, **ALSS**- ALS severity scale, **ALSSQoL-R** - revised ALS-specific Quality of Life questionnaire, **ANCOVA**- analysis of covariance, **APB**- abductor pollicis brevis, **ARSLA**- Association pour la recherche sur la SLA, **ATLIS**- accurate test of limb isometric strength, **ATT**- Advanced Test of ToM, **BAI**- body adiposity index, **BMI**- body mass index, **BNT**- Boston naming test, **CALR**- Calreticulin, **CALSNIC**- Canadian ALS Neuroimaging Consortium, **CC**- corpus callosum, **CDR**- Clinical Dementia Rating, **CGA-NPI**- caregiver-administered Neuropsychiatric Inventory, **CHI3L1**- chitinase-3-like protein 1, **CHI3L2**- chitinase-3-like protein 2, **CHIT1**- chitotriosidase-1, **Cho**- Choline, **CIDP**- chronic inflammatory demyelinating polyneuropathy, **CIS20-R**- checklist individual strength, **CK**- creatinine kinase, **CLIC1**- Chloride intracellular channel protein 1, **CMAP** - compound muscle action potential, **CMCT**- central motor conduction time, **CMT**- Charcot-Marie-Tooth disease, **CNS-BFS**- Center for Neurologic Study-Bulbar Function Scale, **CNS-LS**- Center for Neurologic Study-Lability Scale, **COWAT**- controlled oral word association test, **Cr**- creatinine, **CSF**- cerebrospinal fluid, **CSP**- cortical silence period, **CST**- corticospinal tract, **CypA**- peptidyl-prolyl cis-trans isomerase A, **DCMAP**- distal compound muscle action potential, **D-KEFS**- Delis–Kaplan Executive Function System, **DTI**- diffusion tensor imaging, **DWI**- diffusion-weighted imaging, **EAT**- Emotion Attribution Task, **ECAS**- Edinburgh Cognitive and Behavioural ALS Screen, **ECL**- electrochemiluminescence, **EDB**- extensor digitorum brevis, **ELQ**- Emotional Lability Questionnaire, **EMG**-electromyogaphy, **ERp57**- protein disulfide-isomerase A3, **ET**- Eyes Task, **EURALS**- European Registry of ALS Consortium, **EUROMOTOR**- European

multidisciplinary ALS network identification to cure motor neurone degeneration, **FA**-fractional anisotropy, **FAB**- Frontal Assessment Battery, **FAS**- Phonemic Verbal Fluency Test, **FBI**- frontal behavioral inventory, **FD**- fibre density, **FEV1**- forced expiratory volume, **F-FDG**- fluorodeoxyglucose, **fibs-sw**- fibrillation/sharp-waves, **FLAIR**- Fluid-attenuated inversion recovery, **FM-ADP**- fat mass air displacement plethysmography, **FPs**- fasciculation potentials, **FrsBe**- Frontal Systems Behavior Scale, **FSBS**- Frontal Systems Behavior Scale, **FSS**- fatigue severity scale, **FTD-CDR-SOB**- FTD modified Clinical Dementia Rating Scale Sum of Boxes, **FTLD**- frontotemporal lobar dementia, **FUBP1**- far upstream element-binding protein 1, **FVC**- forced vital capacity, **FWE**-family wise error, **GM**- grey matter, **GNT**- Graded Naming Test, **GSTO1**- glutathione S-transferase omega-1, **HADS**- Hospital Anxiety and Depression Scale, **HDAC4**- histone deacetylase 4, **HDS-R**- Hasegawa Dementia Scale-Revised, **HHD**- handheld dynamometry, **HSC70**- heat shock cognate 71 kDa protein, **IFN**- interferon, **IL**-interleukin, **IRAK4**- Interleukin-1 receptor-associated kinase 4, **JLO**- Judgement of Line Orientation, **JPND**- EU Joint Programme for Neurodegenerative Disease Research, **KOLT**- Kendrick Object Learning Test, **LGVF**- letter guided verbal fluency, **LMN**- lower motor neuron, **MAS**- modified Ashworth scale, **McDESPOT**- multi-component driven equilibrium single pulse observation of T1/T2, **MD**- mean diffusivity, **MDRS**- Mattis Dementia Rating scale, **MDRS-2**- Mattis Dementia Rating scale-Second Edition, **MEP**-maximal static expiratory mouth pressure, **MiND-B**- motor neuron disease behaviour scale, **MIP**- maximal inspiratory pressure, **MiRNAs**- micro-RNAs, **MITOS**- Milano-Torino staging system, **MMSE**- Mini Mental State Examination, **MMT** - manual muscle testing, **MND** – Motor neuron disease, **MNI**- Montreal Neurological Institute, **MOCA**- Montreal Cognitive Assessment, **MR**- magnetic resonance, **MRC**- Medical Research Council Scale for muscle strength, **MRCSS-LL**- Medical Research Council sum score, **MRI**- magnetic resonance imaging, **MRS**- magnetic resonance spectroscopy, **MS**- multiple sclerosis, **MU**-motor unit, **MUNE** - motor unit number estimation, **MUNIX** - motor unit number index, **MUPs**- motor unit potentials, **MUSIX**- motor unit size index, **NAA**- N-acetylaspartate, **NART**- National Adult Reading Test, **NEALS**- Northeast ALS Consortium, **NF-L**- neurofilament light chain, **NI** - neurophysiology index, **NINDS**-Neurological Disorders and Stroke scale, **NISALS**- Neuroimaging Society in Amyotrophic Lateral Sclerosis, **NMR**- nuclear magnetic resonance, **nUHPLC LC-MS**- nano ultra-high

performance liquid chromatography tandem mass spectrometry, **p75ECD**-neurotrophin receptor p75 extracellular domain, **PA28a**- proteasome activator complex subunit 1, **PBA**- pseudobulbar affect, **PBAC**- Philadelphia Brief Assessment of Cognition, **PCR**- polymerase chain reaction, **PDI**- protein disulfide-isomerase, **PEFT**-peak expiratory flow time, **PET**- positron emission tomography, **PGGM**- precentral gyruses gray matter, **PGRN**- progranulin, **PLS** – primary lateral sclerosis, **PMA**-progressive muscular atrophy, **PMC**- primary motor cortex, **pNFH**- Phosphorylated neurofilament heavy chain, **PRDX2**- peroxiredoxin-2, **PRO-ACT**- Pooled Resource Open-Access ALS Clinical Trials, **QoL** – quality of life, **RAVLT**- Rey Auditory Verbal Learning Test immediate and recall, **RCPM**- Raven's Coloured Progressive Matrices, **RD**- radial diffusivity, **RIG**- radiologically inserted gastrostomy, **RMN**-Research Motor Neuron, **ROA2**- Heterogeneous nuclear ribonucleoproteins A2/B1, **ROCFc**- Rey-Osterrieth Complex Figure recall, **RSA**- relative surface area, **rsfMRI**- resting state functional magnetic resonance imaging, **RS-NIRS**- resting state near-infrared spectroscopy, **RSPM**- Raven's Standard Progressive Matrices, **SBMA** - spinal and bulbar muscular atrophy, **SCA**- spinocerebellar ataxia, **SDMT**- symbol digit modality test, **SEIQOL-DW**-Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting, **SF-36**-36-Item short form health survey, **SMA** – spinal muscular atrophy, **SMUAP**- single motor unit action potential, **SNIP**- sniff nasal inspiratory pressure, ***SOD1***- superoxide dismutase 1, **SOP** – Standard operating procedure, **SPECT**- single photon emission computed tomography, **SPO2**- peripheral capillary oxygen saturation, **STA**- Syntax Test for Aphasia, **SVC**- slow vital capacity, **TA**- tibialis anterior, **TBSS**- tract-based spatial statistics, **TDP-43**- TAR DNA-binding protein 43, **TFCE**- threshold-free cluster enhancement, **TiM**- Telehealth in Motor Neuron disease, **TMS** - transcranial magnetic stimulation, **TMT**- Trail Making test, **TNF**- tumor necrosis factor, **ToM**- Theory of Mind, **TROG**- Test for the Reception of Grammar , **TUG**- timed up and go test, **Tw Pdi**- twitch trans-diaphragmatic pressure, **TWBC**- total white blood cell count, **UMN**- upper motor neuron, **VBM**- voxel-based morphometry, **VC**- vital capacity, **VOSP**- Visual Object & Space Perception Battery, **WAIS**- Wechsler Adult Intelligence Scale, **WAIS-R**- Wechsler Adult Intelligence Scale—Revised, **WALS**- Western ALS Consortium, **WCST**- Wisconsin Card Sorting Test, **WMS**- Wechsler Memory Scale–revised, **WRMT**- Words subtest from the Recognition Memory Test, **WVFI** - Written Verbal Fluency Index

CHAPTER 1: INTRODUCTION

A review of the literature

Amyotrophic lateral sclerosis (ALS) is a clinically, genetically and pathologically heterogeneous neurodegenerative condition (1-3). Clinical heterogeneity in ALS is multidimensional owing to variations in upper motor neuron (UMN) and lower motor neuron (LMN) involvement, extra-motor symptoms, age of onset, survival and progression rates. Disease heterogeneity hinders biomarker development (3, 4) which in turn impedes the reliable assessment of candidate drugs in clinical trials (1). Current clinical trials recruit relatively heterogeneous cohorts of symptomatic patients, despite the notion that considerable pathological changes can already be detected at the time of diagnosis (5, 6). The considerable variability in progression rates in ALS is another confounding factor in clinical trial designs (1, 7-10). Imaging and electrophysiological markers have been repeatedly proposed as candidate monitoring markers (11, 12), but it is increasingly clear that a panel of several "wet" and "dry" biomarkers may be required to capture subtle changes over short periods of time (13, 14). The objective of this chapter is the comprehensive and critical evaluation of studies in ALS, focussing on study designs, statistical power, clinical correlations, the sensitivity profile of proposed monitoring markers and their applicability to clinical trials.

Methods

A formal literature search was performed on PubMed using the core search term "amyotrophic lateral sclerosis" combined with each of the following keywords separately: "staging", "monitoring", "outcomes", "clinical", "clinical trials", "electrophysiology", "neurophysiology", "electromyography", "transcranial magnetic stimulation", "motor unit number estimation", "motor unit number index", "positon emission tomography", "single photon emission computed tomography", "magnetic

resonance imaging", "neuroimaging", "imaging", "blood", "urine", "cerebrospinal fluid", "saliva" and "muscle". A supplementary search combined the core search terms with the following keywords: "presymptomatic", "asymptomatic" and "post-mortem". Inclusion criteria included studies investigating imaging, neurophysiological, clinical or biofluid biomarkers in ALS. Animal studies, review papers, opinion pieces, editorials, case reports, and case series were excluded. Only articles written in English and published between January 1980 and August 2018 were reviewed. Based on the above criteria a total of 118 original research papers were selected and reviewed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. **(Figure 1)**

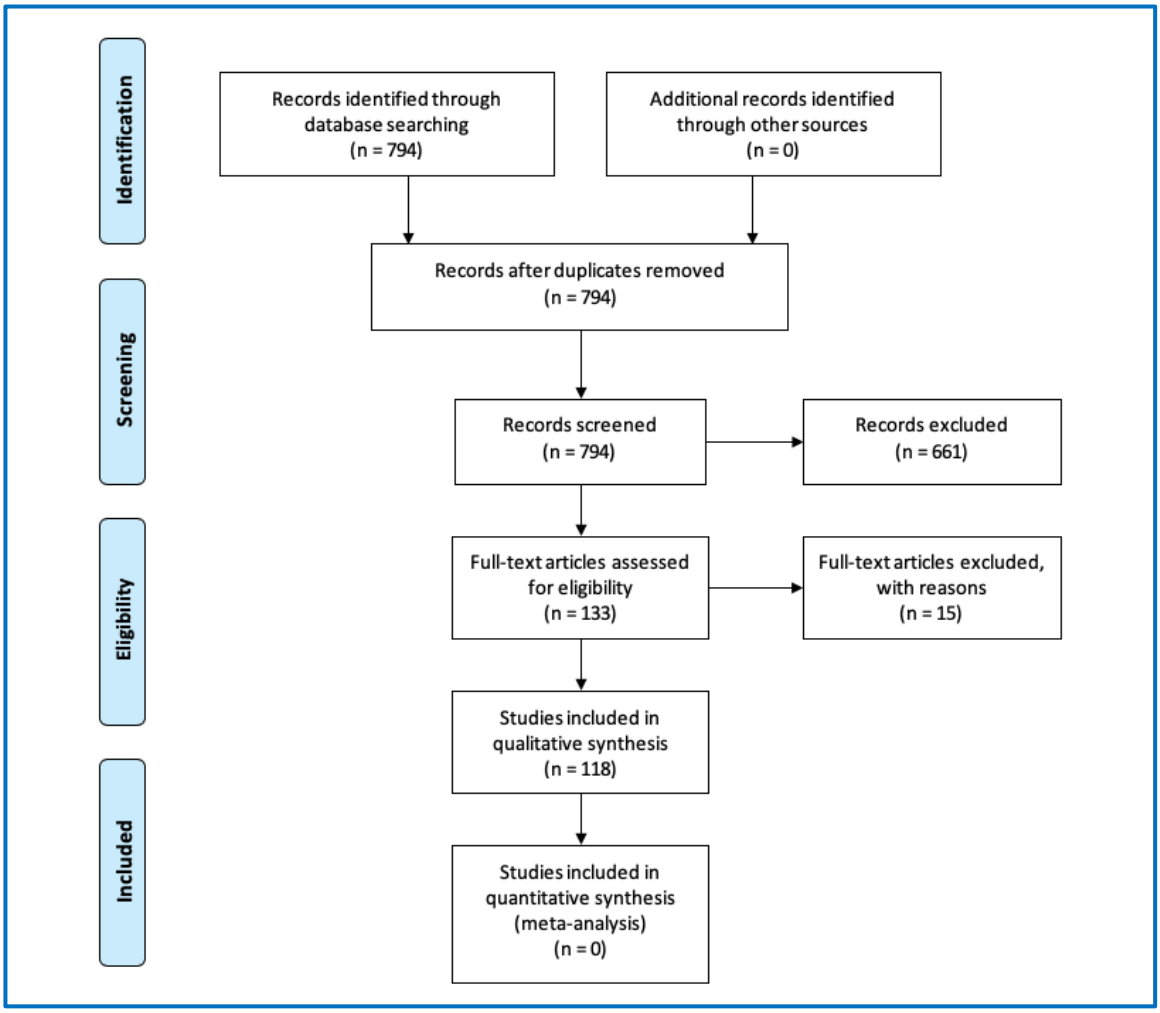


FIGURE 1: *PRISMA flow chart of papers selected for review*

Results

Neuroimaging

Whilst most imaging studies in ALS evaluate cerebral alterations (10), a number of promising spinal studies have now also been published. Spinal imaging has gradually overcome the technical challenges of physiological motion, small cross-sectional dimensions and susceptibility gradients (15-24). The majority of studies in ALS are single-centre studies eliminating the need for cross-platform MR sequence harmonisation and inter-rater reliability tests. Given the low incidence of certain phenotypes such as primary lateral sclerosis (PLS), progressive muscular atrophy (PMA) and spinal and bulbar muscular atrophy (SBMA) however, multisite collaboration is often necessary. (25) The infrastructure, funding and governance of such multicentre collaborations are now established via international consortia like the Neuroimaging Society in Amyotrophic Lateral Sclerosis (NISALS) or the Northeast ALS Consortium (NEALS) (26-29). The need to include disease-controls in addition to healthy controls to describe ALS-specific changes is increasingly recognised (30-32). With few exceptions (33-35), most ALS imaging studies use 3 Tesla platforms and 7 Tesla systems are more commonly used in post-mortem studies (36, 37). Disease progression has been detected across a range of MR imaging metrics including structural (38, 39), diffusion (29, 40), functional (41, 42) and spectroscopy (43, 44) measures. As the majority of longitudinal studies have a two-timepoint design, it is often unclear if specific imaging metrics show linear or exponential changes. The few existing multi-timepoint studies suggest that pathological change is not linear (10). The revised ALS functional rating scale (ALSFRS-r) is the most commonly reported clinical measure (21, 29, 40, 45), with only few imaging studies reporting associations with staging (46) or neuropsychological performance (46, 47) **(Table 1).**

Selection of neuroimaging studies in ALS					
Author(s) and year of publication	**Follow-up interval (months)**	**Number of patients/ Number or controls**	**Clinical assessment batteries/ Functional rating scales**	**Imaging data**	**Summary of findings**
Floeter et al., 2018 (46)	6-18	28/28	ALSFRS-R, letter fluency, FBI, MMSE	DWI, structural (T2)	-progression and propagation over 6 months detected (DTI measures) -DTI measures correlated with ALSFRS-R, King's stage and cognitive measures
Kassubek et al., 2018 (29)	6	67/31	ALSFRS-R	DTI	-progression detected at group level and 27% of individual patients (DTI measures) -FA correlated with ALSFRS-R
Stampfli et al., 2018 (48)	3-6	21/13	ALSFRS-R	T1, DWI	-progression detected (FD values)
Baldaranov et al., 2017 (40)	26	6/6	ALSFRS-R	DTI	-progression detected (FA, AD/RD values) and correlated with progression on ALSFRS-R
Bede et al., 2017 (14)	8	32/69	0	structural,DTI	-progression detected (GM)
de Albuquerque et al., 2017 (21)	8	27/27	ALSFRS-R, UMN scale	structural (T1, T2)	-progression detected (AD, MD) -correlation with ALSFRS-R change
Menke et al., 2017 (45)	24	16/0	ALSFRS-R, UMN score	T1, DTI, rs-Fmri	-progression detected -correlation with ALSFRS-R decline
Simon et al., 2017 (49)	3-6	21/13	ALSFRS-R, MRCSS-LL, MUNE	DTI, structural (T1)	-progression detected (FA values) -correlations with ALSFRS-R change, MUNE, functional disability and strength
Floeter et al., 2016 (38)	6	49/28	ALSFRS-R, FBI, MDRS-2, letter fluency, MMSE, D-KEFS	structural (T1)	-progression detected (ventricular volume)
Schulthess et al., 2016 (28)	6	135/56	ALSFRS-R	rs-Fmri, DTI	-progression detected (functional connectivity) -correlation with

					physical disability
McMillan et al., 2015 (47)	12	20/25	neuropsychiatry	structural (T1)	-hypermethylation protective against progression, correlation with protection of some components of neuropsychological assessment
Steinbach et al., 2015 (50)	3	16/16	ALSFRS-R, neuropsychiatry	DTI	-progression detected
Westeneng et al., 2015 (39)	5.5	112/60	ALSFRS-R	structural (T1)	-progression detected (volume measures) -correlation with ALSFRS-R
Menke et al., 2014 (4)	6	60/36	ALSFRS-R, ACE-R	structural (T1), DTI	-progression detected (GM)
Schuster et al., 2014 (51)	3-15	77/60	ALSFRS-R	structural (T1)	-progression detected (cortical thickness)
Stoppel et al., 2014 (41)	3	40/42	ALSFRS-R, MRC, neuropsych battery	structural, Fmri	-progression detected -correlation with ALSFRS-R and MRC
Verstraete et al., 2014 (52)	5.5	24/19	ALSFRS-R	DTI, structural (T1)	-no progression detected -propagation detected
Ignjatovic et al., 2013 (30)	6	46/26	ALSFRS-R	structural (T1, T2, FLAIR)	-progression detected (hypointensities in PGGM)
Irwin et al., 2013 (31)		143/0	MMSE, LGVF	structural VBM	-no progression on MRI reported
Kolind et al., 2013 (53)	42	30/12	ALSFRS-R, ACE,	mcDESPOT	-progression detected in PLS only
Kwan et al., 2012 (54)	1.26-2.08 years	45/19	ALSFRS-R, finger tapping	T1, DTI	-progression detected (cortical thickness, GM volume)
Keil et al., 2012 (55)	6	24/24	ALSFRS-R, SF36, FAB, MMSE	DTI, structural (T1, T2)	-progression detected (FA values) -correlations with ALSFRS-R, physical and executive function
Menke et al., 2012 (56)	6	24/0	ALSFRS-R	DTI	-progession detected (AD)
Verstraete et al., 2012 (57)	6	45/25	ALSFRS-R	structural (T1)	-no progression reported
Ichikawa et al., 2011 (58)	NA	6/NA	NA	NA	-progression detected, correlated to neuropsychology assessment

van der Graaff et al., 2011 (59)	NA	48/12	ALSFRS-R, finger tapping	DWI	-progression detected
Zhang et al., 2011 (60)	8	17/19	ALSFRS-R	structural (T1), DTI	-progression detected (FA)
Agosta et al., 2009 (61)	9	16/10	ALSFRS	structural (T1)	-progression detected (GM)
Agosta et al., 2009 (62)	9	17/20	ALSFRS	DWI, structural	-progression detected (cord area, cord average FA)
Avants et al., 2009 (63)	5.3	4/4	0	structural (T1)	-progression detected (cortical atrophy)
Blain et al., 2007 (64)	6-12	23/25	ALSFRS-R, ALSS	structural (T2), DWI	-no significant progression detected (DTI measures)
Lule et al., 2007 (42)	6	25/15	ALSFRS-R	Fmri, structural (T1)	-progression detected (activity)
Unrath et al., 2007 (43)	6	11/0	ALSFRS	MRS, T1	-progression detected (NAA, NAA/Cr+Cho)
Rule et al., 2004 (65)	3-12	45/17	0	MRS, structural (T1, T2)	-no clear pattern of progressive change over time (NAA rations)
Suhy et al., 2002 (44)	Every 3 months	28/12	0	MRS, T1, T2	-progression detected (NAA, Cr, Cho)
Block et al., 1998 (32)	24	33/20	0	MRS	-progression detected

TABLE 1*: Selection of neuroimaging studies in ALS*

ACE-R- Addenbrooke's Cognitive Examination-revised, **AD**- axial diffusivity, **ALSFRS-r** - revised ALS functional rating scale, **ALSS**- ALS Severity Scale, **Cho**- Choline, **Cr**- creatinine, **D-KEFS**- Delis–Kaplan Executive Function System, **DTI**- Diffusion Tensor Imaging, **DWI**- Diffusion-weighted imaging, **FA**- Fractional anisotropy, **FAB**- frontal assessment battery, **FD**- fibre density, **GM**- grey matter, **LGVF**- letter guided verbal fluency, **McDESPOT**- multi-component driven equilibrium single pulse observation of T1/T2, **MD**- mean diffusivity, **MDRS-2**- Mattis Dementia Rating scale-Second Edition, **MMSE**- mini mental state examination, **MRCSS-LL**- Medical Research Council sum score, **MRS**- magnetic resonance spectroscopy, **NAA**- N-acetylaspartate , **PGGM**- precentral gyruses gray matter, **RD**- radial diffusivity, **rsfMRI**- resting state functional magnetic resonance imaging, **SF-36**- 36-Item Short Form Health Survey

Electrophysiology

Most neurophysiology studies reviewed are single centre studies, reducing the risk of inter-rater and inter-centre variability (66). Follow-up interval ranges between 7 days (67) and 3 years (68), and up to 7 follow-up time-points have been included in some studies (69, 70). Surprisingly few studies include disease controls such as peripheral neuropathy (69) or benign fasciculation syndrome (71). Clinical assessments performed in conjunction with neurophysiology typically include ALSFRS-r (72), forced vital capacity (FVC) (73), slow vital capacity (SVC) (74), grip strength (75), pinch strength (76) and manual muscle testing (MMT) (76), however, correlations between neurophysiological measures and clinical assessments are seldom reported **(Table 2).** The majority of the neurophysiological studies reviewed focus on upper limb muscles, e.g. abductor pollicis brevis, deltoid, first dorsal interrosseus, extensor digitorum brevis, abductor digiti minimi (69, 72, 73, 77, 78) with relatively few studies evaluating lower limb muscles such as abductor hallicus brevis and tibialis anterior (68, 70, 71, 74). The most commonly reported neurophysiological indices include compound muscle action potential (CMAP) (72, 77), single motor unit action potential (SMUAP) (69), MUNE (73, 79), MUNIX (74, 80), neurophysiology index (NI) (73, 81), TMS measures (70, 76), and axonal excitability (77). Progressive neurophysiological changes have been detected by MUNIX (72, 74, 80), MUNE (69, 72, 76), CMAP (77, 78), NI (81) and TMS measures (70) and allowing for study-design limitations, the consensus is that degenerative changes are not linear.

Selection of electrophysiology studies in ALS					
Author(s) and year of publication	**Follow-up interval (months)**	**Total number of patients/ Total number of controls**	**Neurophysiology modality**	**Target muscle**	**Summary of findings**
Escorcio-Bezerra et al., 2017 (80)	4.3	21/21	MUNIX	tibialis anterior (TA), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles	- progression detected (mean MUNIX)
de Carvalho et al., 2013 (71)	3-6	73/37	FPs, MUPs, fibs-sw, jitter-MU physiology	tibialis anterior	- progression detected
Vucic et al., 2013 (67)	7-100 days	25/30, 35	cortical and axonal excitability-MEP, CMAP-TMS	abductor pollicus brevis	-aim to determine effect of riluzole
Boekestein et al., 2012 (72)	8	18/24	MUNIX, HD-MUNE, CMAP, MUSIX	thenar	- progression detected (MUNE, MUNIX)
Cheah et al., 2012 (77)	3	37/0	CMAP, axonal excitability	abductor pollicis brevis	- progression detected (CMAP)
Ahn et al., 2011 (82)	NA	135/NA	NA	NA	- assymetric progression (MUNE)
Cheah et al., 2011 (81)	3	58/NA	NI, CMAP	abductor digiti minimi and ulnar nerve	- progression detected (NI)
de Carvalho et al., 2010 (73)	6	28/0	NI, CMAP, MUNE	abductor digiti minimi muscles	- progression detected (CSP)
Neuwirth et al., 2010 (74)	15	7/8	MUNIX, CMAP,	abductor pollicis brevis (APB), abductor digiti minimi (ADM), abductor halluces brevis (AHB), and extensor digitorum brevis	- progression detected (MUNIX)

				(EDB)	
Floyd et al., 2009 (70)	18	60/33	TMS, CMCT, MEP	abductor digiti minimi (ADM) and tibialis anterior (TA)	-linear progression detected (TMS threshold, CMCT, TMS amplitude corrected)
Gooch et al., 2009 (76)	NA	64/-1	TMS, MUNE,	NA	- progression detected (MUNE)
Liu et al., 2009 (79)	12	112/12	MUNE, CMAP	Abductor pollicis brevis (APB) and abductor digiti quinti (ADQ)	- progression detected (MUNE), correlated to ALSFRS descent
Albrecht et al., 2004 (69)	11.5	10/25	MUNE, S-MUAP	extensor digitorum brevis	- progression detected (MUNE)
Aggarwal et al., 2002 (68)	36	31/57	MUNE	tibialis anterior, abductor pollicis brevis (APB), deltoid, and first dorsal interosseous muscles	-no progression reported
Arasaki et al., 2002 (83)	NA	NA	MUNE,	extensor digitorum brevis (EDB)	-no progression reported
Wang et al., 2002 (78)	12	20/70	MUNE, SMUP, CMAP, MU loss	thenar	- progression detected (thenar MUNE, CMAP)
de Carvalho et al., 1999 (84)	11.6	NA	CMAP, MEP, TMS	NA	-no progression detected
Chan et al., 1998 (85)	24	NA	motor units	thenar	- progression detected
Felice et al., 1997	12	NA	MUNE,	thenar	-

(86)					progression detected (MUNE)
Yuen et al., 1997 (75)	6	NA	CMAP, MUNE	abductor digiti minimi	- progression detected (MUNE, fiber density)
Swash et al., 1982 (87)	NA	14/NA	single fibre EMG	NA	-no definite progression

TABLE 2: *Selection of electrophysiology studies in ALS*

ADM- abductor digiti minimi, **ADQ**- abductor digiti quinti, **AHB**- abductor halluces brevis, **ALSFRS-r** - revised ALS functional rating scale, **APB**- abductor pollicis brevis, **CMAP**- compound muscle action potential, **CMCT**- central motor conduction time, **CSP**- cortical silence period, **EDB**- extensor digitorum brevis , **EMG**-electromyogaphy, **fibs-sw**- fibrillation/sharp-waves, **FPs**- fasciculation potentials, **FVC**- forced vital capacity, **MU**-motor unit, **MUNE** - motor unit number estimation, **MUNIX** - motor unit number index, **MUPs**- motor unit potentials , **MUSIX**- motor unit size index, NI - neurophysiology index, **SMUAP** - single motor unit action potential, **SNIP**- sniff nasal inspiratory pressure, **SVC**- slow vital capacity, **TA**- tibialis anterior, **TMS** - transcranial magnetic stimulation

Clinical biomarkers and instruments

Robust clinical longitudinal studies in ALS have up to 6 follow-up time points (88-90), the interval between the assessments can be as short as 3 months (91) and the sample size can be as big as several thousands (92, 93) **(Table 3)**. Few multi-timepoint studies include disease controls such as motor neuropathies (89), alternative neuromuscular diseases (94), or neurodegenerative conditions (95). Large, multi-timepoint longitudinal studies invariably suffer from considerable attrition rates, but these are rarely explicitly reported in the manuscript abstracts (10). Detailed genotyping is only available in a minority of studies (46, 96-98). The most widely utilised rating scale in studies is the ALSFRS-r (92, 99, 100) which provides a composite score of bulbar, limb and respiratory dysfunction, and is invariably evaluated in clinical trials (101, 102). Quality of life (QoL) in ALS is increasingly evaluated by disease-specific instruments such as the 40-item ALS assessment questionnaire (ALSAQ-40) or the revised ALS-specific Quality of Life questionnaire (ALSSQoL-R) (103-105). A number of symptom-specific instruments are also commonly used such as the Center for Neurologic Study-Bulbar Function Scale (CNS-BFS), a 21-item self-report scale of bulbar function, and the Center for Neurologic Study-Lability Scale (CNS-LS), a 7-item self-report scale of pseudobulbar affect (PBA) (106). Tapping rates, composite reflex scores, The Penn UMN Score (107), the Modified Ashworth scale (MAS) are often used as proxies of UMN degeneration (106).

In clinical trials, muscle strength is often estimated by handheld dynamometry (HHD) (108), MMT (101), scoring systems such as the Medical Research Council (MRC) Scale for muscle strength (109) and some studies also report limb circumference (110). Respiratory function in ALS is typically monitored by sniff nasal inspiratory pressure (SNIP), SVC, or FVC in addition to measures such as early morning arterial blood gas (ABG) and overnight pulse-oximetry (111, 112). Measures of typing ability (89), tongue movements (113), vital capacity (VC) (114), FVC (115), SNIP (116), and diaphragm amplitude (116) all show progressive longitudinal changes. Nutritional markers such as body mass index (BMI) and lipid profile are now established prognostic indicators (117,

118). Cognitive and behavioural domains are routinely assessed thanks to the availability of validated screening instruments such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (119), the Beaumont Behavioural Inventory (BBI) (120) and the ALS Cognitive Behavioral Screen (ALS-CBS) (121). In contrast to the relentlessly progressive motor deficits of ALS, the trajectory of cognitive and behavioural deficits is less clear due to considerable individual variations, genotype-associated profiles (122, 123), differences in assessment strategies and practice-effects. (124) Many longitudinal neuropsychology studies do not detect progression (96, 125, 126), and some report improvement as a result of practice effects (96).

Selection of clinical studies in ALS				
Author(s) and year publication	**Follow-up interval (months)**	**Number of patients/ Number of controls**	**Clinical assessment batteries/ Functional rating scales**	**Summary of findings**
Andersen et al., 2018 (127)	6-59	20/0	respiratory- SVC, cough peak flow, max inspiratory muscle strength, SNIP, max insufflation capacity	-no progression reported
Poletti et al., 2018 (96)	24	168/0	ECAS	-no progression detected, ECAS scores improved over time
Thakore et al., 2018 (92)	NA	3367/0	ALSFRS-R, ALSFRS, bloods- creatinine, uric acid, CK, albumin, sodium bicarbonate, hematocrit, TWBC	-ALSFRS-R progression detected, preslope and postslope have effects on survival
Andres et al., 2017 (90)	4-21	100/0	ATLIS, ALSFRS, VC	-ATLIS more sensitive to change than ALSFRS and VC
Floeter et al., 2017 (128)	18	NA	ALSFRS-R, letter fluency, FBI	-progression detected (ALSFRS-R, FBI, letter fluency)
Ioannides et al., 2017 (129)	6	44/29	FM-ADP, BMI, BAI, ALSFRS-R	-BMI and BAI not accurate measures of fat mass in ALS
Jakobsson Larsson et al., 2017 (130)	24	36/0	SEIQoL-DW, ALSFRS-R, HADS	-anxiety decreased over time, depression correlated to QOL, QOL remained stable despite physical deterioration
Peter et al., 2017 (131)	3	393/791	BMI, ALSFRS-R	-alterations in body weight present in ALS patients decades before manifestation of symptoms
Quaranta et al., 2017 (132)	NA	NA	respiratory function	-no progression reported
Xu et al., 2017 (94)	6	108/60	ACE-3, FAB, ECAS executive, MoCA, ALSFRS-R, ALS-FTD-Q, MiND-B	-no progression detected
de Bie et al., 2016 (88)	12	10/0	RSA, ALSFRS-R, FVC	-progression detected(RSA and ALSFRS-R)
Gillingham et al., 2016 (97)	9	20/36	ALS-CFB, ALSFRS-R	-no progression reported

Nunes et al., 2016 (133)	3	37/0	BMI, serum albumin, transferrin, total cholesterol	-no progression reported
Rooney et al., 2016 (100)	NA	407/0	ALSFRS-R	-progression detected in ALSFRS-R subscores
Shellikeri et al., 2016 (113)	NA	33/13	kinematic measures of tongue and jaw movement, speaking rate, intelligibility, ALSFRS-R	-progression detected (tongue movement size and speed)
Londral et al., 2015 (89)	2-20	19/26	typing activity, ALSFRS-R	-progression detected (typing activity)
Panitz et al., 2015 (134)	12	51/0	fatigue severity scale (FSS), CIS20-R- subjective fatigue experience, concentration, motivation, activity, ALSFRS-R, MRC, SVC	-progression detected (FSS, CIS20-R), correlated to ALSFRS-R, and ALSFRS-R progression
Proudfoot et al., 2015 (135)	24	61/39	eye tracking- anti saccadic, trail making, visual search tasks, ALSFRS-R, ACE-R, UMN, imaging)	-no progression detected
Atassi et al., 2014 (93)	NA	8635/0	ALSFRS-R, VC	-PRO-ACT database- progression detected (ALSFRS-R and VC)
***Lenglet et al., 2014 (101)**	18	512/0	ALSFRS-R, MMT, SVC	-clinical trial
Mioshi et al., 2014 (136)	6	79/53	MiND-B- apathy, disinhibition, stereotypical behaviour, ACE-R, ALSFRS-R	-no progression reported
Watanabe et al., 2014 (98)	1.7 years	451/0	ALSFRS-R, MRC, MMT	-progression detected (ALSRS-R)
Yamauchi et al., 2014 (137)	Every 6 months	43/30	ALSFRS-R, phrenic nerve conduction study (DCMAP), respiratory function tests (SNIP, FVC), nocturnal pulsed oximetry, MMT	-no progression reported
Elamin et al., 2013 (138)	NA	186/NA	cognitive testing	-progression detected (cognitive function)
Leonardis et al., 2012 (91)	every 3 months	NA/0	ALSFRS-R, Norris-r, AGA, FVC, MIP, MEP, SNIP	-progession detected (respiratory measures)
Mahajan et al., 2012 (114)	NA	362/0	VC	-progression detected (VC)
Roberts-South et al., 2012 (139)	24	16/12	neuropsychology, language, discourse sampling, perfusion	-progression detected (cognitive

			computerized transaxial tomography, pulmonary, clinical	language deficits)
***Duning et al., 2011 (140)**	3	10/32	ALSFRS, clinical neuropsych battery, imaging	-progression detected (DTI)
Pinto et al., 2009 (116)	4-6	49/0	Diaphragm amplitude, ALSFRS-R, MIP, FVC, SNIP, SPO2	-progression detected (Diaphragm amplitude, ALSFRS-R, respiratory measures)
Mendoza et al., 2007 (141)	NA	161/0	MIP, FVC	-no progression reported
***Montes et al., 2007 (142)**	6	31/0	TUG, ALSFRS-R, FVC, MMT	-linear progression detected (TUG) -associated with ALSFRS-R, MMT
Vender et al., 2007 (115)	NA	139/0	FVC	-progression detected (FVC)
Wilson et al., 2005 (143)	NA	55/NA	respiratory- FVC, FEV1, PEFT	-linear progression detected (PEFT)
Beck et al., 2002 (144)	6	78/39	skin water loss	-progression detected (skin water loss)
***ACTS trial., 1996 (102)**	NA	75/NA	ALSFRS	-progression detected (ALSFRS-R), associated with motor and pulmonary function
Marti-Fabregas et al., 1995 (145)	NA	NA	FVC	-no progression detected
Palmowski et al., 1995 (146)	NA	NA	electro-oculography	-not well defined progression
Jablecki et al., 1989 (147)	NA	NA	clinical scores	-no progression reported
Garruto et al., 1988 (95)	NA	31/66	bone mass (wrist radiograph)	-progression detected (bone loss)
Poloni et al., 1983 (148)	NA	NA	VC, Motley index, FEV1	-progression detected (respiratory measures)

TABLE 3: *Selection of clinical studies in ALS*

ACE-3- Addenbrookes Cognitive Examination - Third Edition , **AGA**- arterial gas analyses , **ALS-CFB**- ALS computeried frontal battery, **ALSFRS-r** - revised ALS functional rating scale, **ATLIS**- accurate test of limb isometric strength, **BMI**- body mass index, **CIS20-R**-

checklist individual strength, **CK**- creatinine kinase, **DCMAP**- distal compound muscle action potential, **ECAS**- Edinburgh Cognitive and Behavioural ALS Screen, FBI- frontal behavioral inventory, **FEV1**- forced expiratory volume in 1 second, **FM-ADP**- fat mass air displacement plethysmography, **FSS**- fatigue severity scale, **HADS**- Hospital Anxiety and Depression Scale, **MiND-B**- Motor Neuron Disease Behaviour Scale, **MIP**- maximal inspiratory pressure, **MMT** - manual muscle testing, **MRC**- Medical Research Council Scale for muscle strength, **PEFT**- Peak expiratory flow time , **PRO-ACT**- Pooled Resource Open-Access ALS Clinical Trials, **RSA**- relative surface area, **SEIQOL-DW**- Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting, **SPO2**- peripheral capillary oxygen saturation, **TUG**- Timed Up and Go test, **TWBC**- total white blood cell count, **UMN**- upper motor neuron, **VC**- vital capacity

*indicates clinical trial

Wet biomarkers

Phosphorylated neurofilament heavy chain (pNFH), neurofilament light chain (NF-L), progranulin (PGRN), cytokines, TAR DNA-binding protein 43 (TDP-43), cystatin C, creatinine, micro-RNAs (miRNAs), chitotriosidase-1 (CHIT1), chitinase-3-like protein 1 (CHI3L1), chitinase-3-like protein 2 (CHI3L2) have been evaluated in both research studies (149-156) and clinical trials (154, 157-160) **(Table 4)**. Markers of iron metabolism and ferroptosis are relatively recent domains of ALS biomarker research (161, 162). Most biofluid studies are either serum (154, 160) or CSF studies (156, 163), but urine (164) and skeletal muscle-based (155) studies have now also been published. Quantitative enzyme-linked immunosorbent assay (ELISA) is the most commonly used antibody-based technique (165, 166) which can be performed with one antibody (indirect ELISA), or with two antibodies (sandwich ELISA). Increased CSF (165) and serum (167) pNFH detected by ELISA is thought to be a sensitive marker of axonal degeneration in ALS (150, 156, 168, 169). The specificity of this marker however may be inadequate to reliably differentiate ALS from other neurodegenerative conditions (165, 168). Other antibody-based techniques such as Western blot (150) and electrochemiluminescence (ECL) (151, 155) may improve detection sensitivity and reliability (165). Panels of multiple proteins can be evaluated by multiplex immunoassays such as planar or microbead assays (165). Mass spectrometry based methods using chromatin-immunoprecipitation-based surfaces, two-dimensional gel electrophoresis or high-resolution mass spectrometry have identified cystatin-C and transthyretin as candidate biomarkers (170-172). The longest wet biomarker study reviewed followed patients for 4 years (152). The majority of longitudinal studies have at least 2 follow-up timepoints (153, 164, 173) and one study included 13 follow-up timepoints (157, 174). Large multi-centre trials include as much as 1000 participants (157). One of the most striking shortcomings of existing studies is that very few included disease controls such as Parkinson's disease cohorts, patients with multifocal motor neuropathy with conduction block, Kennedy's disease, chronic inflammatory demyelinating polyneuropathy (CIDP), cervical or lumbar radiculopathy, Charcot-Marie-Tooth disease (CMT), benign fasciculation and cramp syndrome etc. (153, 156, 174). Another limitation of many studies is the lack of comprehensive genotyping (12)

as very few studies report comprehensive screening for ALS-associated mutations (150, 155, 174, 175). Exhaustive clinical profiling, such as medications (152, 156), neuropsychological assessments (150), quality of life indices are rarely reported in longitudinal studies. The majority of studies limit their clinical descriptions to ALSFRS-r, FVC, MRC and Ashworth scores (153, 155, 158). Serum and plasma biomarkers such as creatinine (157, 160), pNfH (149, 174), and micro-RNAs (154), CSF biomarkers such as CHI3L1 (156), tau (158, 159) and cystatin-C (153), and urinary (164) and skeletal muscle (155) biomarkers are some of the promising tools for detecting disease progression. While no progressive changes have been detected in NFL levels, it is likely to be a useful as a diagnostic biomarker (150, 151).

Selection of wet biomarker studies in ALS						
Author(s) and year of publication	**Follow-up interval (months)**	**Number of patients / number of controls**	**Candidate biomarker evaluated**	**Biofluid**	**Assessment method used**	**Summary of conclusion**
***Okada et al., 2018 (160)**	12	57/0	creatinine	serum	NA	-progession detected (creatinine)
Raheja et al., 2018 (176)	NA	NA	microRNAs	serum	NA	-progression detected (miR-136-3p, miR-30b-5p, miR-331-3p, miR-496, miR-2110)
Thompson et al., 2018 (156)	30	49/52	chitotriosidase (CHIT1), chitinase-3-like protein 1 (CHI3L1), and chitinase-3-like protein 2 (CHI3L2), (phosphorylated neurofilament heavy chain) Pnfh	CSF	nano ultra-high performance liquid chromatography tandem mass spectrometry (nUHPLC LC-MS/MS), ELISA	-progression detected (CHI3L1)
Di Pietro et al., 2017 (155)	NA	14/24	micro-RNAs-MIR206, MIR208B, MIR499	skeletal muscle	quantitative real time PCR, Western blot analysis	-progression detected (MIR208B, MIR499, MIR206, HDAC4)
Gaiani et al., 2017 (177)	36	94/82	ALSFRS-R, NFL	CSF	enzyme-linked immunosorbent assay (UmanDiagnostics AB)	-NFL may have role as a biomarker
Murdock et al., 2017 (178)	Every 6-12 months	119/35	leukocytes	blood	flow cytometry	-progression detected (immune cells), associated with ALSFRS-R
Shepheard et al., 2017 (164)	NA	54/45	urinary p75ECD	urine	sandwich ELISA	-progression detected (urinary p75ECD), correlated with ALSFRS-R

van Ejik et al., 2017 (157)	NA	1241/0	creatinine	plasma	NA	-progression detected (plasma creatinine), correlated to ALSFRS-R, muscle strength, mortality
Waller et al., 2017 (154)	3	22/0	microRNAs, miR-17-5p, miR-223-3p, miR-24	serum	Qiagen miScript-based Qpcr	-progression detected (mir-206, mir-143-3p, mir-374b-5p)
Lu et al., 2016 (152)	48	95/88	CK, ferritin, tumor necrosis factor (TNF)–a, and interleukin (IL)–1b, IL-2, IL-8, IL-12p70, IL-4, IL-5, IL-10, and IL-13, IL-6, IFN-Y	plasma	multiplex electrochemiluminescence immunoassay	-no defined progression
Steinacker et al., 2016 (179)	24	125/28	neurofilament light chain (NF-L), progranulin (PGRN), S100	serum, CSF (baseline only)	ELISA, electrochemiluminescence (ECL) immunoassay, ECLIA Elecsys (Roche, Penzberg, Germany)	-no progression reported
Gibson et al., 2015 (180)	12	80/0	CK	NA	NA	-no progression detected
Gray et al., 2015 (163)	24	41/14	CSF- glucose, lactate, citric acid, ethanol	CSF	H-NMR	-no progression reported
Lu et al., 2015 (151)	36	167/78	neurofilament light chain (NFL)	serum, blood, CSF	electrochemiluminescence immunoassay	-no progression detected
McCombe et al., 2015(149)	27	98/61	pNFH	serum	NA	-progression detected (pNFH)
Lu et al., 2014 (174)	36	136/104	neurofilament heavy chain-phosphoform	plasma	ELISA	-progression detected (NfH)
***Levine et al., 2012 (159)**	6	28/0	tau, pNFH	CSF	ELISA	-progression detected (tau)
Verstraete et al., 2012 (175)	NA	219/100	TDP-43	plasma	sandwich ELISA	-no defined progression
Nardo et al.,	6	94/64	PRDX2,	blood PBMC	2D-DIGE,	-no

2011 (173)			GSTO1, CLIC1, HSC70, CypA, PDI, ERp57, CALR, PA28a, IRAK4, FUBP1, ROA2, actinNT, TDP-43		mass spectometry	progression reported
***Levine et al., 2010 (158)**	12	20/0	tau, pNFH	CSF	ELISA	-progression detected (tau)
Wilson et al., 2010 (153)	24	44/60	cystatin C	CSF, plasma	quantitative enzyme linked immunosorbent assay (ELISA)	-progression detected (cystatin C)

TABLE 4: *Selection of wet biomarker studies in ALS*

2D-DIGE- two-dimensional fluorescence difference gel electrophoresis, and IL- interleukin, **CALR**- Calreticulin, **CK**- cytokines, **CLIC1**- Chloride intracellular channel protein 1, **CypA**- Peptidyl-prolyl cis-trans isomerase A, **ERp57**- Protein disulfide-isomerase A3, **FUBP1**- Far upstream element-binding protein 1, **GSTO1**- Glutathione S-transferase omega-1 , **HSC70**- Heat shock cognate 71 kDa protein, **IFN**- interferon, **IRAK4**- Interleukin-1 receptor-associated kinase 4, **MiRNAs**- micro-RNAs , **NF-L**- neurofilament light chain, **NMR**- Nuclear Magnetic Resonance, **nUHPLC LC-MS**- nano ultra-high performance liquid chromatography tandem mass spectrometry, **p75ECD**- neurotrophin receptor p75 extracellular domain, **PA28a**- Proteasome activator complex subunit 1, **PCR**- polymerase chain reaction, **PDI**- Protein disulfide-isomerase, **PGRN**- progranulin , **pNFH**- Phosphorylated neurofilament heavy chain , **PRDX2**- Peroxiredoxin-2, **ROA2**- Heterogeneous nuclear ribonucleoproteins A2/B1, **TDP-43**- TAR DNA-binding protein 43, **TNF**- tumor necrosis factor

*indicates clinical trial

Studies of asymptomatic mutation carriers

Current clinical trials only recruit symptomatic cases despite accruing evidence that ALS has a long presymptomatic phase. (5) Imaging studies of asymptomatic mutation carriers have consistently confirmed disease-specific cerebral and spinal cord changes prior to symptom onset (181-184) indicating that this disease-phase may represent a crucial window for therapeutic or neuroprotective intervention. Please see **Table 5** for further detail. The majority of asymptomatic studies assess a single time-point, as opposed to the longitudinal tracking of asymptomatic carriers of ALS-causing mutations (46). While the overwhelming majority of asymptomatic studies focus on *C9orf72* hexanucleotide carriers (183, 185-187), no prognostic markers have been validated to predict whether single patients will develop ALS or FTD. Compared to imaging studies, strikingly few asymptomatic neurophysiology studies have been undertaken (68). Studies of asymptomatic ALS-causing mutation carriers have enormous potential for academic research and may pave the way for asymptomatic pharmaceutical trials (5, 181).

Selection of imaging studies investigating changes in asymptomatic mutation carriers			
Author, year of publication and reference	**Sample size (presymptomatic patients/healthy controls)**	**Mutation**	**Summary of findings**
Bertrand et al., 2018 (188)	41/39	*C9orf72*	-Cognitive, structural, and microstructural alterations are detected -focal atrophy of left supramarginal gyrus -WM alterations
Wen et al., 2018 (185)	38/29	*C9orf72*	-white matter abnormalities in 10 tracts with neurite density index, in 5 tracts with DTI -changes detected for GM cortical analysis
Lee et al., 2017 (183)	15/46	*C9orf72*	-functionally compensated brain volume and connectivity deficits; similar, but less severe, than those in symptomatic phase
Papma et al., 2017 (189)	18/15	*C9orf72*	-decline in cognitive functioning, white matter integrity, and gray matter volumes
Menke et al., 2016 (181)	12/12	*C9orf72 and SOD1*	-increased functional connectivity among the earliest detectable brain abnormalities
Carew et al., 2011 (182)	24/29	*SOD1*	-changes in neurometabolite ratios in the C-spine
Ng et al., 2008 (184)	8/13	*SOD1*	-abnormal diffusivity at the posterior limb of internal capsule

TABLE 5: *Selection of imaging studies investigating changes in asymptomatic mutation carriers*

Discussion

Clinical trials currently evaluate the efficacy of candidate drugs using the ALSFRS-r, muscle strength assessment tools such as MMT, respiratory function indices such as FVC, SVC and SNIP, neurophysiological measures and survival (22, 26, 127, 190, 191). These measures however primarily reflect late-stage functional impairment and are not indicative of early stage pathology. Brain and spinal cord imaging has been evaluated as early-stage biomarkers with both diagnostic and monitoring potential (22, 26, 192). Longitudinal imaging studies are superior to cross-sectional studies as they readily detect dynamic structural and functional changes and may elucidate compensatory processes (10, 14, 26, 28, 41, 193, 194). The emergence of multi-timepoint study designs (14, 195) enable the characterisation of anatomical propagation patterns (196) and provide invaluable temporal insights into the disease trajectory of late-stage ALS. Inter-scan intervals as short as 3 months can detect longitudinal changes (14, 26, 40). Many imaging studies make use of multiple magnetic resonance (MR) metrics which is particularly useful in establishing an optimal panel of monitoring markers (26). The core neuroimaging signature of ALS, irrespective of the disease-stage, includes corticospinal tract (197, 198), corpus callosum (199) and motor cortex degeneration (200). Atrophy in frontotemporal regions has been primarily associated with neuropsychological deficits (201-203) and linked to hexanucleotide repeats in *C9orf72*. (123, 204) Several studies have indicated that white matter degeneration can be detected relatively early in the course of ALS with restricted further progression over time, whereas grey matter pathology shows relentless progression in the symptomatic phase of the disease (4, 14, 26). In addition to structural imaging studies, connectivity-based, metabolic, peripheral nerve, and, whole body muscle imaging have contributed to our understanding of longitudinal changes (195, 205-207).

Needle electromyography and nerve conduction studies play an important clinical role in ruling out alternative conditions and confirming a suspected diagnosis of ALS. Despite variations in local protocols, neurophysiological tests are recognised as

objective, reliable and cost-effective tests of neuromuscular dysfunction have also been repeatedly proposed as markers of disease progression (73, 208). CMAP is generated by depolarisation of muscle fibres through the stimulation of a single nerve, where amplitude reductions are interpreted as loss of motor axons (209, 210). While CMAP measurements capture decline, it is confounded by variations in temperature, limb positioning and electrode placement (74, 211). CMAP-derived measures such as MUNE and MUNIX are now extensively utilised to characterise progressive changes in ALS. MUNE estimates motor neuron numbers, and may detect the rate of motor neuron loss, making it a more reliable method of appraising disease progression than CMAP (212, 213). However, its early-phase sensitivity has been questioned, as its use is limited to distal muscles, and the technique requires considerable training, especially for inter-rater and multi-site comparisons (209, 214). TMS allows the characterisation of upper motor neuron dysfunction, and may be particularly useful in detecting progressive changes (70, 209).

Functional rating-scales are often the monitoring instruments of choice in clinical trials (73), as they are easy to administer, cost-effective to utilise and have acceptable inter- and intra-rater reliability profiles (215). The most widely used rating scale in clinical studies is the ALSFRS-r. Despite its ease of administration, it has considerable limitations, as it may be disproportionately influenced by LMN dysfunction, does not account for laterality or asymmetry of symptoms, omits cognitive impairment, and may be affected by medications (14, 99, 190, 216).

Proteomics, metabolomics and lipidomics have seen significant advances in ALS research and CSF and serum markers are now used in longitudinal academic and pharmacological studies (161). Potential biomarkers for the detection of disease progression include serum and plasma biomarkers such as creatinine (157, 160), pNfH (149, 174), and micro-RNAs (154), CSF biomarkers such as CHI3L1 (156), tau (158, 159) and cystatin-C (153), and urinary (164) and skeletal muscle (155) biomarkers.

Prediction analyses

Age at symptom onset (217), BMI (117), bulbar involvement (218), cognitive impairment (219), *C9orf72* genotype status (122), respiratory insufficiency (220), 'definite ALS' by the El Escorial criteria (221), and functional disability (222) are the most commonly cited determinants of poor prognosis in ALS. SNIP (223) and less commonly used measures such as twitch trans-diaphragmatic pressure (Tw Pdi) (224) and maximal static expiratory mouth pressure (MEP) were shown to be good predictors of ventilator-free survival (224). A combined panel of several clinical, wet and dry biomarkers is likely to offer the most accurate prognostic information (20, 26, 221, 222, 225). While cerebral (222, 226, 227) and spinal (20) imaging measures have been repeatedly linked to survival outcomes, these have not been utilised in a clinical setting. Neurophysiological variables, such as phrenic nerve stimulation outcomes (228) and biofluid markers, such as pNFH and NFL (151, 179, 229-231) are also thought to be accurate predictors.

Patient stratification

Attempts to enrol patients in the early stages of the disease are hampered by the universally long diagnostic delay in ALS (232). Patient stratification in trials is typically based on site of onset (233), instead of other variables which have an established prognostic impact (112, 234). Admixed patient cohorts within a trial may hamper the ability to detect how different phenotypes and genotypes may exhibit a different response to a candidate drug (235-237). The stratification of heterogeneous cohorts is now aided by the development of validated staging systems, such as the King's (238), Milano-Torino (MITOS)(239) or the Fine'til 9 (FT9) (240) staging systems. The King's Staging system is based on the number of body regions affected, and the presence of nutritional or respiratory failure (238). The MITOS staging system is based on the ALSFRS-r, and is particularly sensitive to changes in later stages of the disease (241, 242). However, none of these staging systems account for cognitive or behavioural changes (241). Pathological staging systems suggest a four-stage model of ALS based

on anatomical patterns of pTDP-43 load (243, 244). This system has now been validated by in vivo neuroimaging studies (245) and signals that accurate pathological staging and patient stratification may be possible based on neuroimaging (194, 245).

International consortia

Only few ALS centres maintain dedicated biobanking facilities to store and process molecular markers in human biofluid locally. Similarly, relatively few centres are in a position to generate sufficient number of MRI and neurophysiology data sets of rare phenotypes to make meaningful inferences in a single centre setting. Brain and tissue banks are also challenging to establish, maintain and fund, despite their invaluable contribution to ALS research (246-248).

Biospecimen samples are also often collected during clinical trials, and discarded after negative outcomes, despite their enormous potential for biomarker discovery (161). One of the most important achievements of biomarker development efforts is the establishment of national and international research consortia such as Association pour la recherche sur la SLA (ARSLA), Neuroimaging Society in ALS (NISALS), Research Motor Neuron (RMN), Canadian ALS Neuroimaging Consortium (CALSNIC), EU Joint Programme for Neurodegenerative Disease Research (JPND), European multidisciplinary ALS network identification to cure motor neurone degeneration (EUROMOTOR) which maintain vital biobanking facilities, registries, data repositories for multicentre data interpretation (27, 249). Clinical trial networks are also increasingly recognised as valuable platforms for multisite data collection and interpretation as they operate with carefully standardised protocols. Consortia such as the European Registry of ALS (EURALS) Consortium, the Western ALS (WALS) Consortium and the Northeast ALS (NEALS) Consortium are other examples (250). NEALS is one of the largest consortia with over 100 member sites from the US, Canada, Mexico, Italy, Lebanon and Australia (251). EURALS coordinates research studies and

clinical trials relying on population-based European registries and include centres from Scotland, England, Netherlands, Spain, Ireland, Serbia, Italy, France and Germany (246, 252, 253). ALS research consortia promote patient-oriented research, maintain biofluid, imaging and DNA banks, and have the potential to translate scientific advances into pragmatic clinical interventions.

Telehealth

Novel trends in data collection include telemedicine-based technologies, wearable sensors and mobile phone applications (235). The continuous collection of data via telephone or telemedicine applications such as the Telehealth in Motor Neuron disease (TiM) system circumvent the inconvenience of patients and caregivers traveling long distances for research appointments (254). Once local data-protection and governance guidelines are complied with, information uploaded from these systems can be made available to healthcare professionals of multidisciplinary teams in real time (254). The feasibility of telehealth for ALS patients via live video-conferencing has also been evaluated (255) and is considered a particularly promising clinical and research platform (254, 255). A number of cognitive-behavioural screening tools have also been adapted for phone administration (256) including modified versions of the ALS Cognitive Behaviour Screen (ALS-CBS), the Controlled Oral Word Association Test (COWAT), the Center for Neurologic Study-Lability Scale (CNS-LS) and found to be statistically equivalent to face-to-face assessments (256). Performance on other tests however, such as the telephone versions of the ALS-Frontal Behavioral Inventory (ALS-FBI) caregiver interview and the Written Verbal Fluency Index (WVFI) was not equivalent to clinic-based assessments (256). The continued development of telephone and internet-enabled devices are likely to provide further insights to physical, cognitive and behavioural changes (256).

Conclusions

While clinical biomarkers are undeniably useful, neuroimaging, neurophysiology and biofluid measures are particularly promising biomarker candidates. It is likely that a combination of biomarkers will be required to facilitate accurate early-stage prognostication for individual ALS patients. The validation of reliable “wet” biomarkers and “dry” biomarkers including neuroimaging biomarkers will enable improved diagnosis and the detection of subtle progressive changes. This would allow precision stratification of heterogeneous patient cohorts in clinical trials and improve existing prediction algorithms.

CHAPTER 2: MOTOR AND EXTRA-MOTOR CHANGES IN ALS

The imaging signature of ALS

Voxel based morphometry (VBM), an automated grey matter technique, typically shows focal degenerative cortical patterns in the motor regions corresponding to functional disability. (4, 257) There are different patterns of atrophy in patients with cognitive impairment and those without, with cognitively impaired patients showing extensive atrophy in extra-motor regions, particularly in the frontotemporal areas. (258, 259) In addition to cortical grey matter changes, subcortical grey matter changes have also been identified. Atrophy has been detected in the caudate nucleus, hippocampus, thalamus and nucleus accumbens. (4, 39, 202) Furthermore, cerebellar degeneration has also been consistently described. (260) Diffusion tensor imaging allows the assessment of white matter integrity through the quantitative evaluation of the diffusivity characteristics. (261) Altered diffusivity is typically detected in the corticospinal tracts and corpus callosum in ALS. (11) Extensive white matter pathology has been described beyond these white matter regions, especially in patients with cognitive impairment.(258, 262)

Extra-motor pathology in ALS

The perception of amyotrophic lateral sclerosis (ALS) as a disease selectively affecting the motor system has been challenged in the past two decades (194, 263, 264). Extensive frontal and temporal involvement is now universally recognised. Sensory (265), autonomic (266), olfactory (267) and visual dysfunction are rarely described but have been observed and documented by clinicians (194, 268). ALS is largely characterised as an anterior brain disorder (268), however, neuropathology, neuropsychology and neuroimaging studies have also shown evidence of pathology in the parietal and occipital cortices of ALS patients (269).

Patches of reactive astrocytes have been found in the occipital cortex of sporadic and familial ALS patients (270). Significant tau pathology has been found to involve the occipital cortex (271). TDP-43 pathology has been found in the occipital lobes in post mortem studies (272) **(Table 6).** Visuospatial function is a key component of several neuropsychological instruments such as the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (119) and Addenbrooke's Cognitive Examination-revised (ACE-R)(273), but can also be assessed by the Raven's Coloured Progressive Matrices (RCPM) (274). Impairment in visuospatial ability has been identified in a small proportion of patients (119, 275) with some studies, reporting up to 24% of patients with evidence of visuospatial dysfunction (273, 276) **(Table 7)**. Structural (257, 277), diffusion (278), functional MRI (263, 279) as well as susceptibility weighted imaging (SWI) (280) and MR spectroscopy (281) have captured pathology in the parietal and occipital lobes **(Table 8)**. Cortical thinning of parietal (282) and occipital (204, 283) areas has been shown. Impaired activation (263), perfusion alterations (279), metabolic changes (284), and reduced NAA/Cho ratio (281) are some of the occipital changes detected in ALS. Despite evidence that a subset of patients may have of pathology in the parietal and occipital region of the brain, patients are rarely tested for visuospatial impairment such as simultanagnosia, which if present, may impact on patients' quality of life (QoL).

Postmortem evidence of occipital pathology in ALS				
Author(s) and year of publication and reference	**Total number of patients/ Total number or controls**	**Other assessments**	**Methods**	**Conclusion**
Behrouzi et al., 2016 (271)	80/0	0	paraffin section, immunostaining	-tau pathology' in entorhinal cortex, hippocampus, temporal cortex, frontal cortex and occipital cortex
Brettschneider et al., 2014 (285)	39/0	MMSE, FAS, BNT	Immunohistochemistry	-four neuropathological patterns in bvFTD consistent with hypothesis that pTDP-43 pathology can spread sequentially and propagate along axonal pathways
Chen-Plotkin et al., 2009 (286)	11/?	0	Immunohistochemistry	-progranulin mutation-bearing cases had significant reduction in progranulin protein in cerebellum and occipital cortex, but not frontal and temporal cortices
Hiji et al., 2008 (287)	13/0	0	Immunohistochemistry	-p62-positive inclusions in subcortical white matter in frontal and parietal lobes, and to lesser extent in temporal lobe -rarely found in occipital lobe
Petri et al., 2006 (288)	5/5	0	in situ hybridization histochemistry	-prefrontal and temporal cortex has significantly reduced mRNA expression of the alpha1-subunit
Nagy et al., 1994 (270)	15/16	NA	protein immunocytochemistry	-patches of reactive astrocytes not restricted to motor cortex -found in the gray matter in frontal, temporal, inferior parietal, cingulate, occipital, and motor cortices

TABLE 6: *Postmortem evidence of occipital pathology in ALS*

MMSE- Mini Mental State Examination**, FAS-** Phonemic Verbal Fluency Test **, BNT-** Boston naming test

Neuropsychology studies of visuospatial function in ALS				
Author(s) and year of publication and reference	**Total number of patients/ Total number or controls**	**Neuropsychology assessment batteries**	**Other assessments**	**Conclusion**
Crockford et al., 2018 (289)	161/80	ECAS	BBI	-significant differences between patients and controls on all subtests of the ECAS except visuospatial functioning
Masuda et al., 2018 (290)	131/151	ACE-R	MMSE, ALSFRS-R	-in ACE-R total score and almost all subdomain scores except for visuospatial domain, there was significant difference between ALS and control
Burkhardt et al., 2017 (125)	40/49	ECAS	FAB	-controls show significantly higher overall score and subdomain scores of ECAS, except for visuospatial function and fluency
Marjanovic et al., 2017 (275)	27/82	MMSE, RSPM, WAIS-R, Stroop, phonemic and category fluency, ROCFc, RAVLT, BNT	SNIP, ALSFRS-R	-bulbar involvement and more extensive neuropsychological impairment (including executive, visuospatial, and memory problems) were main features of *SOD1* negative group
Trojsi et al., 2016 (291)	22/15	executive, verbal comprehension, visuospatial, behavioural, QoL, QOL, ToM (EAT, ATT, ET)	ALSFRS-R, UMN	-correlation analysis revealed that EAT and ET positively correlated with education, memory prose, visuo-spatial performances
Niven et al., 2015 (292)	40/40	ECAS	neuropsychology-executive, fluency, language, memory, visuospatial	-one patient showed impairment only in the visuospatial domain
Oh et al., 2014 (293)	166/0	executive, attention, language, calculation, visuospatial, memory	K-MMSE, CGA-NPI, CDR	-visuospatial dysfunction was only in a small number of patients
Wei et al., 2014 (273)	145/50	ACE-R	MMSE	-patients had broad range of cognitive impairment domains, including visuospatial ability (24.13%),

Yoshizawa et al., 2014 (276)	25/0	STA, FAB, RCPM, HDS-R	ALSFRS-R	-of 25 patients, six (24%) had visuospatial dysfunction (RCPM score < 24)
Abrahams et al., 2013 (119)	48/40	ECAS	0	-6% showed abnormal ALS Non-specific scores
Wicks et al., 2009 (294)	58/35	WVFI, WCST, GNT,TROG, KOLT, WRMT,VOSP, JLO	HADS, ALSFRS-R, NART, RSPM, FrsBe, ELQ	-memory, receptive language, and visuospatial perception spared
Rippon et al., 2006 (295)	40/80	executive, attention, language, visuospatial, memory	MMSE	-demented patients showed predominant impairment in free recall, executive function, and naming, with preserved of attention, psychomotor speed, and visuospatial function

TABLE 7: *Neuropsychology studies of visuospatial function in ALS*

ALSFRS-R- Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, **ATT**- Advanced Test of ToM, **BNT**- Boston naming test, **CDR**- Clinical Dementia Rating, **CGA-NPI**- caregiver-administered Neuropsychiatric Inventory, **EAT**- Emotion Attribution Task, **ECAS**- Edinburgh Cognitive and Behavioural ALS Screen, **ELQ**- Emotional Lability Questionnaire, **ET**- Eyes Task, **FAB**- Frontal Assessment Battery, **FrsBe**- Frontal Systems Behavior Scale, **GNT**- Graded Naming Test, **HADS**- Hospital Anxiety and Depression Scale, **HDS-R**- Hasegawa Dementia Scale-Revised, **JLO**- Judgement of Line Orientation, **KOLT**- Kendrick Object Learning Test, **MMSE**- Mini Mental State Examination, **NART**- National Adult Reading Test, **QOL**- quality of life , **RAVLT**- Rey Auditory Verbal Learning Test immediate and recall, **RCPM**- Raven's Coloured Progressive Matrices, **ROCFc**- Rey-Osterrieth Complex Figure recall, **RSPM**- Raven's Standard Progressive Matrices, **SNIP**- Sniff nasal-inspiratory pressure, **STA**- Syntax Test for Aphasia, **ToM**- Theory of Mind, **TROG**- Test for the Reception of Grammar , **VOSP**- Visual Object & Space Perception Battery, **WAIS-R**- Wechsler Adult Intelligence Scale—Revised, **WCST**- Wisconsin Card Sorting Test, **WRMT**- Words subtest from the Recognition Memory Test, **WVFI**- Written Verbal Fluency Test

Imaging evidence of occipital pathology in ALS					
Author(s) and year of publication and reference	**Total number of patients/ Total number or controls**	**Clinical assessment batteries/ Functional rating scales**	**Imaging modality**	**Imaging modality details**	**Summary of findings**
Christidi et al., 2018 (296)	17/22	ALSFRS-R	structural (t1, t2 FLAIR)	3	-significantly reduced GM density in frontal, temporal, parietal/occipital and cerebellar regions
Ferraro et al., 2018 (279)	30/33	MMSE, PBAC, ALSFRS-R	structural (T1), perfusion	3	-bvFTD has reduced frontotemporal CT -hypoperfusion encompassing orbitofrontal and temporal cortices -hyperperfusion in motor and occipital regions
Li et al., 2018 (297)	38/35	ALSFRS-R	rsf-MRI	3	-extra-motor areas displaying extensive FCD alterations encompassed the temporal cortex, insula, cingulate gyrus, occipital cortex, and inferior parietal lobule
Machts et al., 2018 (298)	158/86	ALSFRS-R, MOCA, executive, visuospatial, verbal, behaviour	structural (T1)	3	-prefrontal, premotor, motor, and occipital cortical thickness related to patients' general cognitive abilities
Schonecker et al., 2018 (299)	58/19	FTD-CDR-SOB, MMSE	structural (T1)	1.5, 3	-mutation carriers show a significant volume reduction of thalamus -most striking in the occipital, temporal and prefrontal subregion of thalamus
Agosta et al., 2017 (204)	86/22	ALSFRS-R, UMN score, MMSE, exective function, verbal memory, naming, behaviour	structural (T1, T2, FLAIR), DTI, rsf-MRI	3	-*C9orf72* patients have occipital cortical thinning
Geevasinga et al., 2017 (300)	20/0	MMSE, MRC, UMN	structural (T1), rsf-MRI	3	-reduced connectivity in frontal regions -increased connectivity in occipital regions
Loewe et al.,	64/38	ALSFRS-R,	structura	3	-changes in connectivity

2017 (205)		executive, memory, language, visuospatial	l (T1), rsf-MRI		across temporo-occipital cortex
Trojsi et al., 2017 (301)	22/18	ALSFRS-R, ACE-R, executive, working memory, sustained attention, verbal comprehension, visuospatial, behaviour, BDI2	HARDI, structural (T2)	3	-larger areas of decreased GFA found in patients with bulbar phenotype in bilateral associative fiber tracts, such as superior and inferior longitudinal, inferior fronto-occipital and uncinate fasciculi
Zhang et al., 2017 (302)	25/25	ALSFRS-R	f-MRI	3	-significantly reduced LGI in right occipital cortex
Kopitzki et al., 2016 (303)	31/30	ALSFRS, neuropsych battery-memory, executive, visuospatial	rs-NIRS, DTI	3	-significant correlation between frontal and temporo-occipital homotopic rs-FCs
Ma et al., 2016 (304)	20/20	ALSFRS-R	rsf-MRI	3	-increased fALFF in right middle frontal gyrus -decreased fALFF in left middle occipital gyrus
Zhou et al., 2016 (305)	43/44	ALSFRS-R	rsf-MRI	3	-significant increase of DC in the left cerebellum posterior lobes, bilateral cerebellum crus, bilateral occipital poles, right orbital frontal lobe, and bilateral prefrontal lobes -significant decrease of DC in the bilateral primary motor cortex, bilateral sensory motor region, right prefrontal lobe, left bilateral precuneus, bilateral lateral temporal lobes, left cingulate cortex, and bilateral visual processing cortex
Evans et al., 2015 (306)	17/36	MMSE, cognition, working memory, visual verbal test	structural (T1), DWI	3	-regression analyses related impaired cognitive flexibility to gray matter atrophy in inferior frontal and insular regions, and to

					reduced FA in white matter projections in the inferior fronto-occipital and uncinate fasciculi and corpus callosum
Prell et al., 2015 (280)	27/30	ALSFRS-R	SWI	1.5	-signal alterations in corpus callosum, corticospinal tract and in subgyral regions of frontal, parietal, temporal, occipital and limbic lobes
Zhu et al., 2015 (307)	22/22	ALSFRS-R, MMSE, MOCA, Stroop, WCST, FAB	structural (T1), rsf-MRI	3	-decreased gray matter volume in bilateral precentral gyri -increased ALFF values in the right parahippocampal gyrus, left inferior temporal gyrus, left anterior cingulate gyrus, right superior frontal gyrus, and left middle occipital gyrus
Crespi et al., 2014 (308)	22/55	ALSFRS-R, language, memory, executive, behaviour	structural (T1, T2, FLAIR), DTI	NA	-early emotion recognition deficit -associated with microstructural changes in ventral associative bundles connecting occipital, temporo-limbic and orbitofrontal regions in right hemisphere
Hartung et al., 2014 (309)	30/37	ALSFRS-R, MMSE, FAB	structural (T1)	1.5	-widespread white matter intensity increases in corticospinal tracts, corpus callosum, sub-central, frontal and occipital white matter tracts and cerebellum
Pagani et al., 2014 (284)	57/40	FSBS, MMSE, WCST, TMT, WMS,WAIS, Stroop, letter and category fluency,Raven's progressive coloured matrices, HADS	PET	F-FDG	-hypometabolism in frontal, motor, and occipital cortex -hypermetabolism in midbrain, temporal pole, and hippocampus

Bede et al., 2013 (310)	27/42	executive function, language, behavior,memory, phonemic fluency, semantic fluency, visuospatial function	structural (T1), DTI	3	-females show a trend of higher age-adjusted cortical thickness in right parieto-occipital and left mid-frontal regions -males have higher cortical thickness in the left lingual and left superior temporal regions
Meoded et al., 2013 (278)	30/17	D-KEFS, MDRS, ALSFRS-R	structural (T1), DWI	3	-changes in diffusion metrics of white matter long association tracts suggest loss of integrity of the networks connecting fronto-temporal areas to parietal and occipital areas contributes to cognitive impairment
Mezzapesa et al., 2013 (283)	29/20	ALSFRS-R, Stroop, SDMT, FAS	structural (T1, T2, FLAIR)	1.5	-cortical thinning in bilateral precentral gyrus, bilateral middle frontal gyrus, right superior temporal gyrus and right occipital cortex
Thorns et al., 2013 (311)	14/14	ALSFRS-R, MRC, NINDS reflex score	structural (T1)	1.5	-regions of cortical thinning include superior and inferior parietal lobule, angular and supramarginal gyrus, insula, superior frontal, temporal and occipital regions
Verma et al., 2013 (281)	21/10	ALSFRS-R	structural (T1)	3	-NAA/Cho ratio lower in caudate, lingual gyrus, supramarginal gyrus, and right and left superior and right inferior occipital lobes
Agosta et al., 2012 (282)	44/26	ALSFRS-R	structural (T1)	1.5	-bilateral cortical thinning of superior and inferior temporal and parietal regions, and medial and lateral occipital areas
Bede et al., 2012 (257)	33/44	ALSFRS-R, behavioural, executive, memory, letter fluency, attention, visuospatial,	structural (T1, FLAIR)	3	-cortical ALS pathology extends beyond motor cortex, affects frontal, occipital and temporal regions

		language			
Boxer et al., 2011 (312)	10/23	0	structural (T1)	1.5, 3	-mild parietal and occipital lobe atrophy
Sarro et al., 2011 (313)	16/15	MMSE, verbal memory, learning, visuospatial, spatial memory, attention, executive, behaviour	DWI	1.5	-performances at tests assessing attention and executive functions correlated with DT MR imaging metrics of the corpus callosum, CST, and long association WM tracts bilaterally, including cingulum, inferior longitudinal, inferior fronto-occipital, and uncinate fasciculi
Agosta et al., 2010 (314)	24/20	ALSFRS-R	structural (T2), DTI	1.5	-extramotor increase of MD in the frontal, temporal, and occipital lobes
Abrahams et al., 2004 (263)	28/18	HADS, NART, Raven's SPM score, executive function tests, memory function tests, language function tests, visuoperceptual function tests	f-MRI	1.5	-significantly impaired activation in middle and inferior frontal gyri and anterior cingulate gyrus, in addition to regions of parietal and temporal lobes

TABLE 8: *Imaging evidence of occipital pathology in ALS*

ACE-R- Addenbrookes Cognitive Examination, **ALSFRS-R**- Revised Amyotrophic Lateral Sclerosis Functional Rating Scale, **D-KEFS**- Delis–Kaplan Executive Function System, **DTI**- diffusion tensor imaging, **DWI**- diffusion-weighted imaging, **FAB**- frontal assessment battery, **FAS**- Phonemic Verbal Fluency Test, **F-FDG**- fluorodeoxyglucose, **FLAIR**- Fluid-attenuated inversion recovery, **FSBS**- Frontal Systems Behavior Scale, **FTD-CDR-SOB**- FTD modified Clinical Dementia Rating Scale Sum of Boxes, **HADS**- hospital anxiety and depression scale, **MDRS**- Mattis Dementia Rating scale, **MMSE**- Mini Mental State Examination, **MOCA**- Montreal Cognitive Assessment, **MRC**- Medical Research Council Scale for muscle strength, **MRI**- magnetic resonance imaging, **NART**- National Adult Reading Test, **NINDS**- Neurological Disorders and Stroke scale, **PBAC**-

Philadelphia Brief Assessment of Cognition, **PET**- positron emission tomography, **Rsf-MRI**- resting state functional MRI, **RS-NIRS**- resting state near-infrared spectroscopy, **SDMT**- symbol digit modality test, **TMT**- Trail Making test, **UMN**- Upper Motor Neuron, **WAIS**- Wechsler Adult Intelligence Scale, **WCST**- Wisconsin Card Sorting Test, **WMS**- Wechsler Memory Scale–revised

CHAPTER 3: HYPOTHESES

In imaging studies of ALS, motor pathology has been extensively characterised, but there is established clinical evidence of extensive extra-motor pathology in ALS. While the main neuroimaging signature of ALS, regardless of the disease-stage, includes corticospinal tract (197, 198), corpus callosum (199) and motor cortex degeneration (200), atrophy in frontotemporal areas has been primarily associated with neuropsychological deficits (201-203) and linked to hexanucleotide repeats in C9orf72. (123, 204) Autonomic (266), olfactory (267), sensory (265) and visual dysfunction are infrequently described in ALS. To that end, the objective of this research project is to use multi-parametric MR imaging to explore both cerebral motor and extra-motor pathology in ALS in comparison to healthy controls (HCs). Based on my systematic literature review (Chapter 1), my hypothesis is that the multi-parametric analyses of cerebral structural and diffusion metrics will reveal the following changes in ALS in comparison to healthy controls:

1) focal cortical changes in the motor cortex;

2) altered diffusivity in corticospinal tracts and corpus callosum;

3) changes in extra-motor regions.

Accordingly, the secondary objective of this study is to examine which extra-motor grey and white matter regions are affected.

CHAPTER 4: METHODS

Recruitment of patients and controls

ALS patients were diagnosed according to the El-Escorial-revised criteria (315) and recruited from the ALS outpatient clinic in Beaumont Hospital, Dublin. Patients were excluded if they had metal implants which were incompatible with MRI, if they reported severe claustrophobia, or if they had developed respiratory symptoms such as orthopnoea which made them unable to tolerate an MRI scan. A subset of patients declined to participate in the research study for other reasons. Forty-five patients attended an appointment at St James' Hospital, Dublin in order to undergo multimodal imaging and detailed clinical evaluation. Details of the imaging protocols used and the clinical assessments are presented below.

Cross-sectional data was obtained and analysed for these patients. A subset of patients continued to participate in the research study for the collection of longitudinal data for future analysis. Fifteen age- and gender-matched healthy controls were also recruited to be scanned in this period, adding to the data repository of healthy controls who have been scanned with the same sequence parameters in previous years. Healthy controls were excluded if they had severe claustrophobia or anxiety which would make them unlikely to be able to tolerate an MRI scan. They were also excluded if they had any neurological illness, a family history of ALS, uncontrolled hypertension, cardiac conditions or diabetes mellitus which could contribute to vascular findings and affect the interpretation of our analyses. Two ALS patients who attended the appointment were unable to tolerate MRI due to orthopnoea and claustrophobia. One healthy control who attended the appointment was unable to tolerate imaging due to claustrophobia. **(Figure 2)**

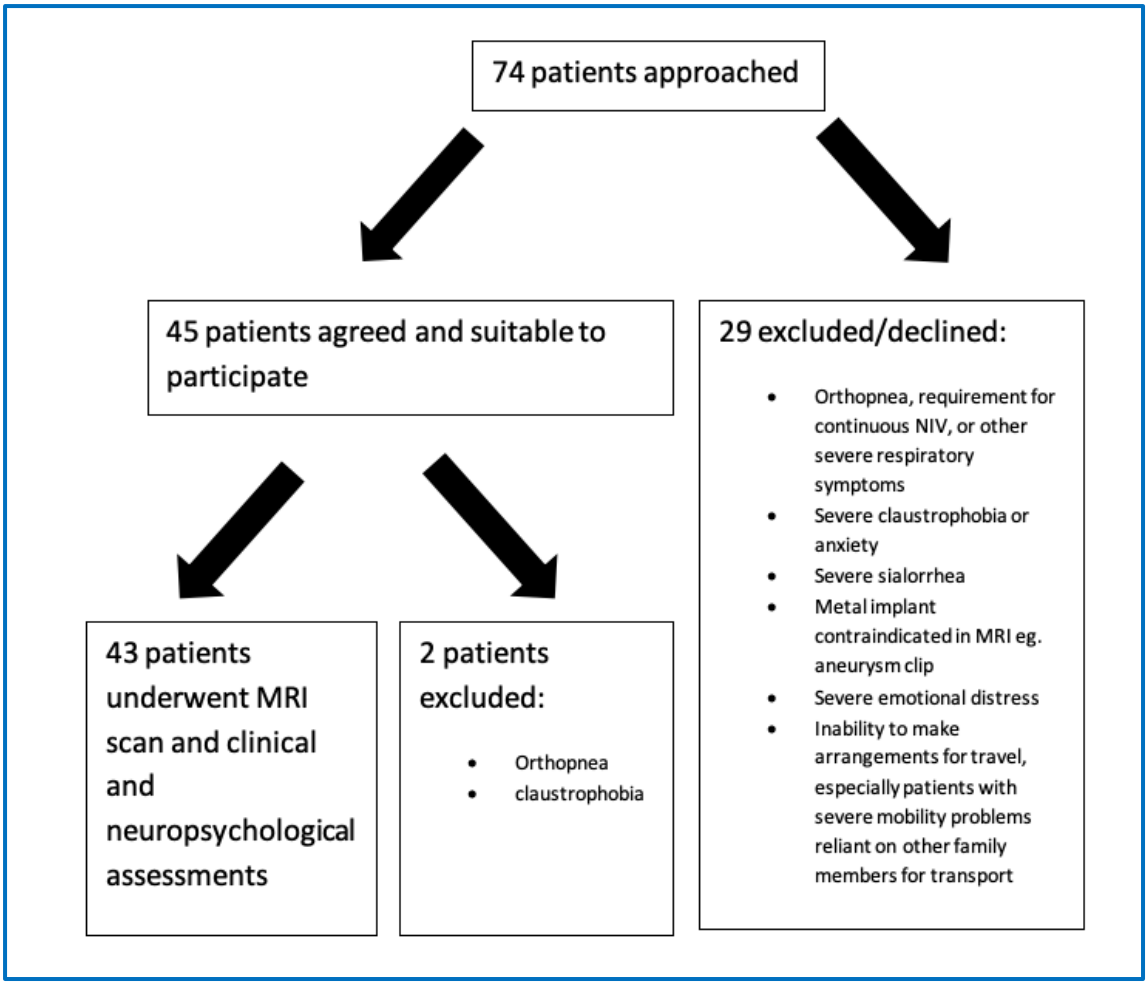


FIGURE 2: *Flow chart of inclusion and exclusion criteria for recruitment into research study*

For the imaging analyses, to boost the sample size of patients and controls recruited by myself I was given access to a cohort of patients previously scanned by my supervisor. In total, the imaging data of 85 ALS patients and 76 controls was analysed to be presented herein.

Methods of clinical assessment

Newly recruited patients were evaluated by an extensive battery of clinical, neuropsychological and behavioural assessments. Clinical assessments performed on patients included the ALS functional rating scale (revised) (ALSFRS-R) (99), Penn Upper Motor Neuron (UMN) Score (316), the Medical Research Council (MRC) scale for muscle strength, and finger tapping. Patients were screened for emotional lability with the Center for Neurologic Study- Lability Scale (CNS-LS) (317), and then given a more detailed assessment with the Emotional Lability Questionnaire (ELQ) (318). The ELQ was completed by both patients and caregivers. Behaviour was assessed with the Frontal Systems Behavior Scale (FrsBe) (319) and the Hospital Anxiety and Depression Scale (HADS) (320). The FrSBe was also completed by both patients and caregivers. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS)(119) was used for neuropsychological assessment.

Neuroimaging methods

A total of 85 ALS patients and 76 healthy controls were included in our neuroimaging analyses. A dual approach was undertaken to evaluate anatomical patterns of pathology in ALS. First, standard whole brain analyses were performed to assess great and white matter alterations. Subsequently, region-of-interest (ROI) analyses were undertaken to assess the integrity if specific anatomical regions and the distribution profile of imaging metrics in individual patients.

Whole brain grey matter analyses

Grey matter pathology was evaluated by voxel-based morphometry using FMRIB's FSL suite. (321, 322) Standard pre-processing steps were used, including skull-removal (BET), motion-corrections and tissue-type segmentation. Grey-matter partial volume data were aligned to the MNI152 standard space using affine registration. Subsequently, a study-specific GM template was created to which the grey matter images from each subject were non-linearly coregistered. For group comparisons, permutation based non-parametric inference was used with the threshold-free cluster enhancement (TFCE) method controlling for age and gender. Statistical significance was thresholded at $p<0.01$ to highlight cortical regions affected in ALS compared to controls.

Region of interest grey matter analyses

Additional region-of-interest (ROI) grey matter analyses were carried our based on atlas defined cortical segmentation. Montreal Neurological Institute (MNI) space-registered cortical ROIs were generated for the bilateral precentral gyrus, the bilateral postcentral gyrus, the occipital, parietal, frontal and temporal lobes. Cortical masks for the occipital, parietal, frontal and temporal lobes were generated based on the anatomical labels of the MNI atlas (323) and the masks for the precentral and postcentral gyrus were defined based on the labels of Harvard-Oxford (HO) probabilistic cortical atlas. The HO atlas was developed based on the segmentation and affine-registration of 21 healthy male and 16 healthy female subjects to MNI152 space. (324, 325) For each ROI mask the 2x2x2 mm 25% probability maps were utilised of the original atlas labels. These labels, containing values of '1', were then used to mask the study specific GM template in MNI space to generate study specific ROIs. The resulting ROI masks are shown in **Figure 3**.

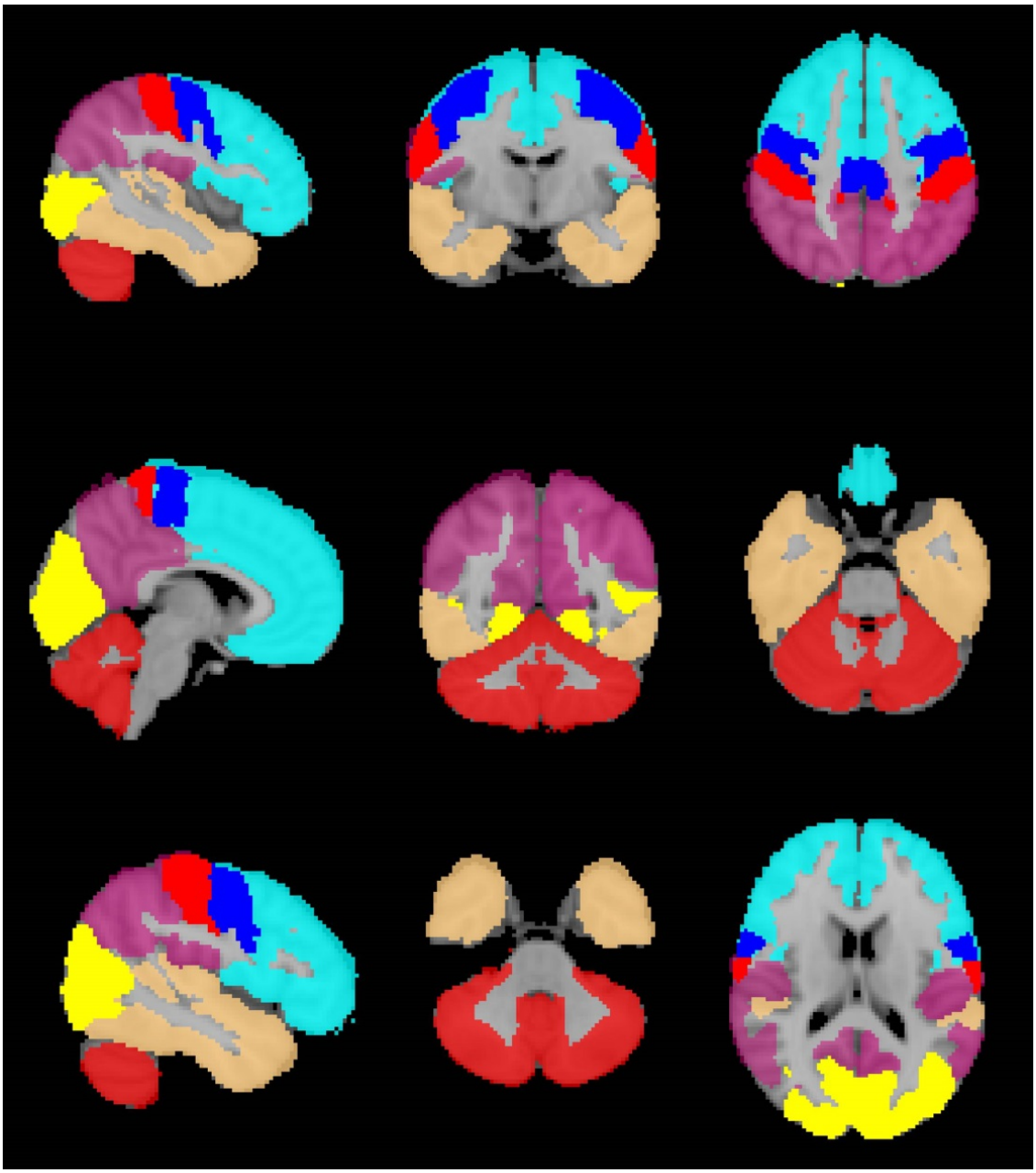


FIGURE 3*: Cortical grey matter mask: aquamarine- frontal lobe, navy blue- pre-central gyrus, red- post-central gyrus, purple- parietal region, yellow- occipital region, copper- temporal lobe, maroon- cerebellum*

Subsequent to ROI generation, average partial volumetric values were retrieved from the above ROIs from each study participant. Assumptions of normality, linearity and homogeneity of variances were verified before analyses of covariance (ANCOVA) were carried out. ROI volumes were included as dependent variables, and study group allocation (ALS vs HC) as the independent variable. Age and gender were used as covariates. A p-value ≤0.05 was considered significant. Results were summarised in a

table including the estimated marginal means of volumes for each ROI, standard error, and between-group ANCOVA significance. Boxplots of volumes were generated to highlight volumetric differences between the study groups and histograms generated to illustrate the distribution of data in the study groups.

Whole brain white matter analyses

Following Eddy current corrections and skull removal a tensor model was fitted to the raw diffusion data to generate maps of fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD). The tract-based statistics (TBSS) pipeline of the FSL image analysis suite was utilised for non-linear registration and skeletonisation of each subject's images. FA, MD, RD and AD images were merged into a single 4D image file and a mean FA mask was created. Similarly to the VBM analyses, permutation-based non-parametric inference was used for the voxelwise comparison of diffusivity parameters between ALS patients and healthy controls using design matrix-defined contrasts which included age and gender as covariates. The threshold-free cluster enhancement (TFCE) method was applied and results considered significant at a $p < 0.05$ TFCE family-wise error (FWE).

Region of interest white matter analyses

The study specific white matter skeleton was masked by atlas-defined labels for the corticospinal tracts, corpus callosum, the temporal, parietal, occipital and frontal lobe to generate study specific ROIs **(Figure 4)**.

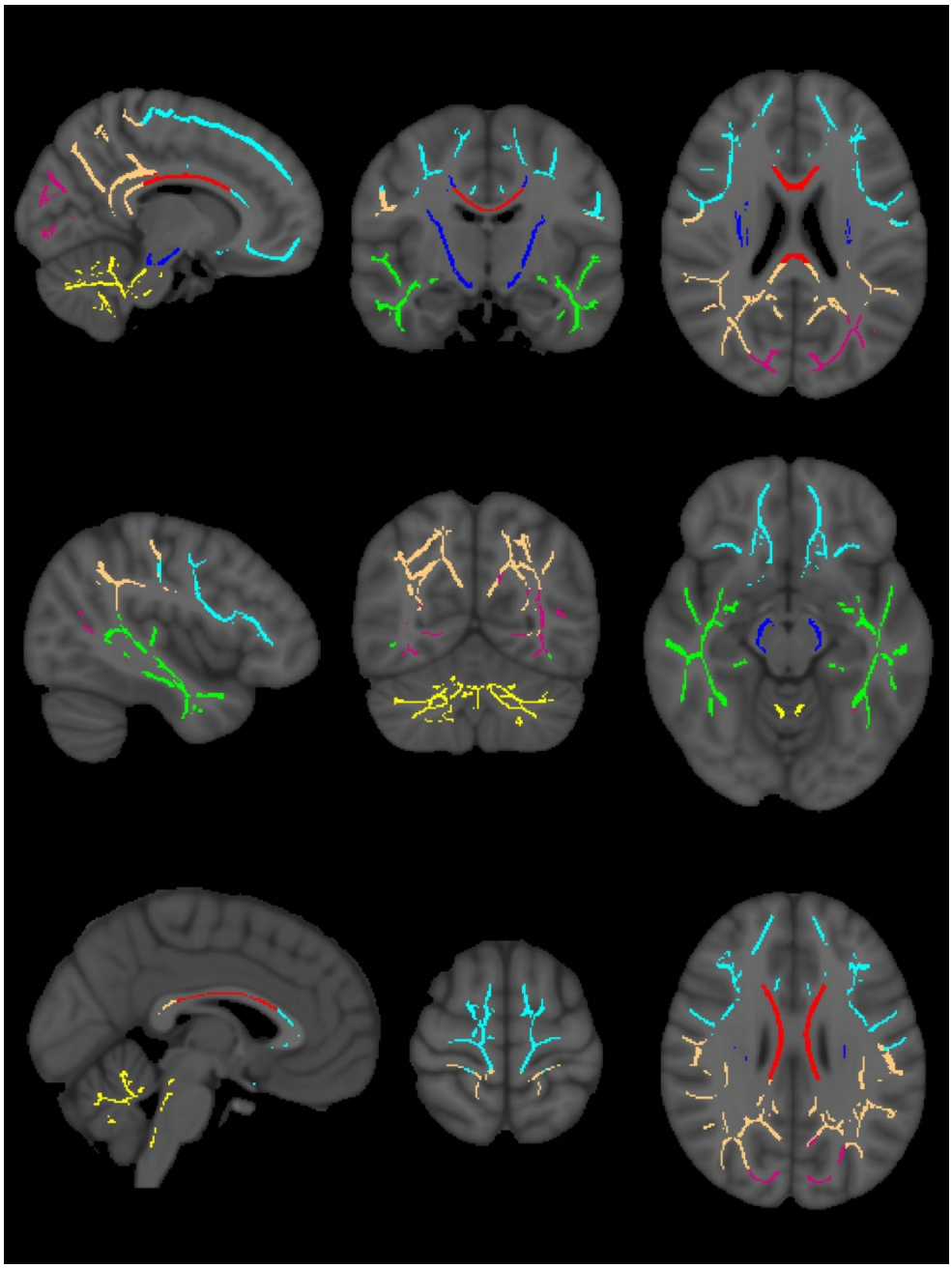


FIGURE 4: *White matter Regions of interest (ROIs): aquamarine- frontal lobe, copper- parietal, purple- occipital, yellow- cerebellum, navy blue- corticospinal tracts, green-temporal lobe, red- corpus callosum*

Diffusivity metrics; axial diffusivity, fractional anisotropy, radial diffusivity was retrieved form the above ROIs and study group differences were evaluated in analyses of covariance, accounting for age and gender. For illustrative purposes values from individual subjects were shown in boxplots and histograms.

CHAPTER 5: RESULTS

The clinical profile of study participants

Demographic and clinical details

Demographic and clinical details were carefully recorded for all study participants as age, gender and education impact on imaging metrics (326) **(Table 9).** The demographic and clinical details of the newly recruited patients and controls are presented in this chapter. There is marked sexual dimorphism in ALS which contributes to disease heterogeneity (310). The site of onset, age of onset, cognitive profile and prognosis has also been shown to vary with gender (218, 310, 327). Therefore gender, as well as age (326), handedness (328, 329) and years of education(330, 331) which will also have an impact on the imaging signature were recorded and corrected for.

Demographic and clinical details of newly recruited ALS patients and Healthy Controls					
	ALS patients	Healthy Controls	p value comparison to controls	Range	Confidence interval
N	43	14	NA	NA	NA
Gender (male/female)	30/13	7/7	0.182	NA	NA
Handedness (right/left/ambidextrous)	42/1/0	13/0/1	0.383	NA	NA
Age (years, SD)	62.3(8.9)	59.7(13.1)	0.475	30.3-84.5	(-10.2), (4.8)
Disease duration (months, SD)	29.7(35.1)	NA	NA	5-168	NA
Education (years, SD)	12.6 (2.9)	14.6 (3.2)	0.028	6-17	(-3.9), (-0.23)
ALSFRS-R mean	38.3 (6.6)	NA	NA	15-48	NA
ALSFRS-R bulbar score	9.7 (2.9)	NA	NA	2-12	NA
ALSFRS-R limb score	17.6 (5.5)	NA	NA	0-24	NA
ALSFRS-R respiratory score (max 12)	11.1 (1.5)	NA	NA	6-12	NA

ALSFRS-r: range of total ALSFRS is 0–48; range of bulbar scores is 0–12, range of limb scores is 0–24, range of respiratory scores is 0–12.

TABLE 9: *Demographic and clinical details of newly recruited ALS patients and Healthy Controls*

With regards to presenting symptoms, the majority of patients presented with either bulbar or upper limb symptoms. A large subset of the patients presented with lower limb symptoms. One patient presented with a mixture of upper and lower limb symptoms and 2 patients presented with weight loss **(Table 10)**. Sixteen patients had asymmetry in presenting symptoms, with 11 patients presenting with symptoms in the left limbs versus 5 patients presenting with symptoms in the right limbs. Eleven patients presented with symptoms in both sides **(Table 11).**

Symptom onset in the ALS cohort		
Presenting symptom	**Frequency**	**Percent**
bulbar	14	32.6
upper limb	14	32.6
lower limb	12	27.9
upper and lower limbs	1	2.3
weight loss	2	4.7
Total	43	100.0

TABLE 10: *Symptom onset in the ALS cohort*

Site of onset of presenting symptoms		
Site of onset of presenting symptoms	**Frequency**	**Percent**
both	11	25.6
right	5	11.6
left	11	25.6
bulbar	14	32.6
weight loss	2	4.7
Total	43	100.0

TABLE 11: *Site of onset of presenting symptoms*

Comorbid conditions were also systematically recorded in our ALS cohort. Eight patients were noted to have hypertension, 4 patients had hypercholesterolemia, 3 had cardiac conditions such as atrial fibrillation or ischaemic heart disease and 2 had hypothyroidism. Other comorbid conditions included polymyalgia, coeliac disease, hereditary haemochromatosis, diabetes mellitus, sinusitis, Barrett's oesophagus, and idiopathic thrombocytopenic purpura. Two patients were noted to have a history of psychiatric conditions including depression, anxiety and bipolar disorder. Twenty-five ALS patients had no comorbid disorders. Healthy controls were excluded if they had a history of uncontrolled hypertension, known cardiac conditions and type I diabetes mellitus to minimise the confounding effect of potential vascular changes. Healthy controls were also excluded if they had a family history of ALS or frontotemporal

dementia. Two controls had treated hypercholesterolemia, and two had treated hypothyroidism.

Five ALS patients were using intermittent non-invasive ventilation (NIV) and 3 had a radiologically inserted gastrostomy (RIG) tube in situ at the time of the MRI scan. A thorough medication history was also taken from each participating ALS patient. 74% of patients (32 patients) were on Riluzole. Three patients were on Baclofen and 2 patients were on Tizanidine for spasticity. Seven patients were on Amitrptyline. Two patients were on quinine. In addition to Riluzole and medications for symptomatic management, patients were also on medications for the management of pre-existing comorbidities.

Family history of neurological conditions was also systematically recorded. Two patients had a second degree relative diagnosed with ALS All other patients had no known family history of ALS. Eleven patients had a family history of dementia, with one of these patients reporting Alzheimer's disease in a 3rd degree relative. Twelve patients had a family history of Parkinson disease and 2 had a family history of multiple sclerosis (MS). There was no known family history of spinal and bulbar muscular atrophy (SBMA) or frontotemporal lobar dementia (FTLD) in our patient cohort.

Smoking and alcohol history were also recorded in patients and controls. Only 2 patients and 2 controls reported to be active smokers at the time of the imaging. A previous history of smoking was noted in 19 patients and 2 controls. Twenty-two patients and 10 controls were non-smokers. The average pack year history of smoking was 6.12 in patients, and 0.83 in controls and thep value was not significant (0.126). The average alcohol consumption in a week of patients was 6.16 units and 5.04 units in controls and the p value was not significant (0.646).

The Clinical profile of patients

Functional disability was assessed in all 43 patients using the ALSFRS-R. The average total ALSFRS-R score for patients was 38.3, ranging from 15-48 and only one patient had a normal total ALSFRS-R score **(Table 12)**. The average bulbar score is 9.7, ranging from 2-12, the average limb score is 17.6, ranging from 0-24 and the average respiratory score is 11.1, ranging from 6-12. Twenty-three patients had abnormal bulbar scores, 38 patients had abnormal limb scores, and 18 patients had abnormal respiratory scores. Thirty patients had abnormal upper limb scores and 4 of these patients had normal lower limb scores. Thirty-four patients had abnormal lower limb scores and 8 of these patients had normal upper limb scores. Five patients who were scanned died within a year of the first MRI scan.

ALSFRS-R total and domain scores				
ALSFRS-R Domain	**Average**	**Range**	**Number of patients with abnormal score**	**Percentage of patients with abnormal score**
Bulbar score	9.7	2-12	23	53.5
Upper limb score	8.8	0-12	30	69.8
Lower limb score	8.6	0-12	34	79.1
Total limb score(upper and lower limb score)	17.6	0-24	38	88.4
Respiratory score	11.1	6-12	18	41.9
Total score	38.3	15-48	42	97.7

ALSFRS-r: range of total ALSFRS is 0–48; range of bulbar scores is 0–12, range of upper limb scores is 0-12, range of lower limb scores is 0-12, range of total limb scores is 0–24, range of respiratory scores is 0–12.

TABLE 12: *ALSFRS-R total and domain scores*

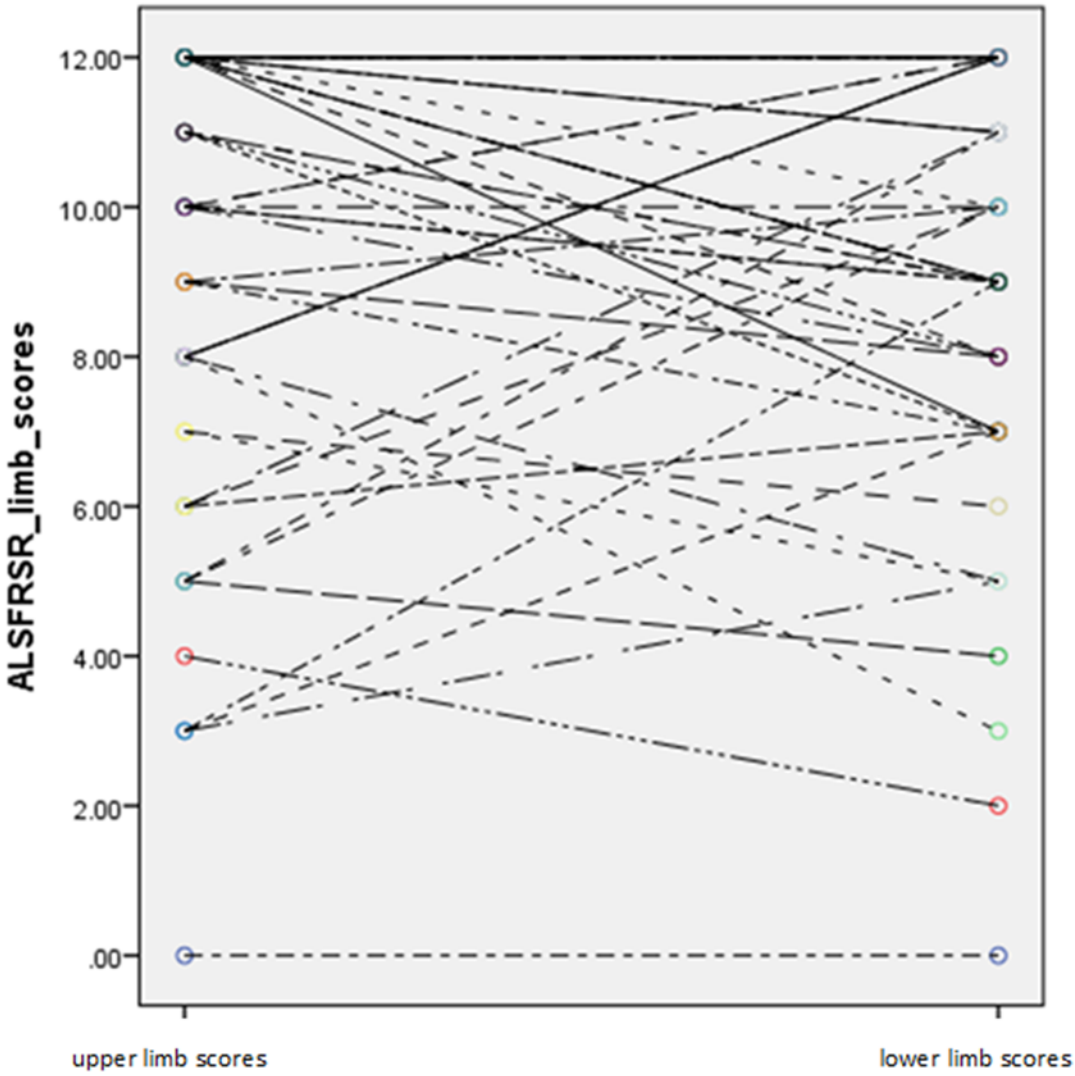


FIGURE 5: *ALSFRS-R upper and lower limb scores*

Thirty-one patients were assessed with the Penn Upper Motor Neuron Score. Four patients had a normal total UMN score of 0 and 27 had an abnormal total UMN score. Twenty-two had an abnormal bulbar score, 20 had an abnormal right upper limb score, 22 had an abnormal left upper limb score, 21 had an abnormal right lower limb score and 25 had an abnormal left lower limb score.

Grey and white matter changes on 'whole-brain' analyses

Patterns of grey matter atrophy

On VBM, significant focal GM changes were observed between ALS patients and controls (**Figure 6)**. in both motor and extra-motor regions. Consistent with the hallmark imaging signature of ALS, considerable motor cortex atrophy was observed at $p < 0.01$ TFCE FWE. In the frontal lobe additional superior frontal, inferior frontal, and orbitofrontal changes were observed (**Figure 6).** Bitemporal changes were also observed including the parahippocampal region, right superior temporal gyrus and both temporal poles. The left insula and pars opercularis region which is consistent with Broca's region also exhibit atrophy. No cerebellar or occipital changes were observed at $p < 0.01$ TFCE FWE.

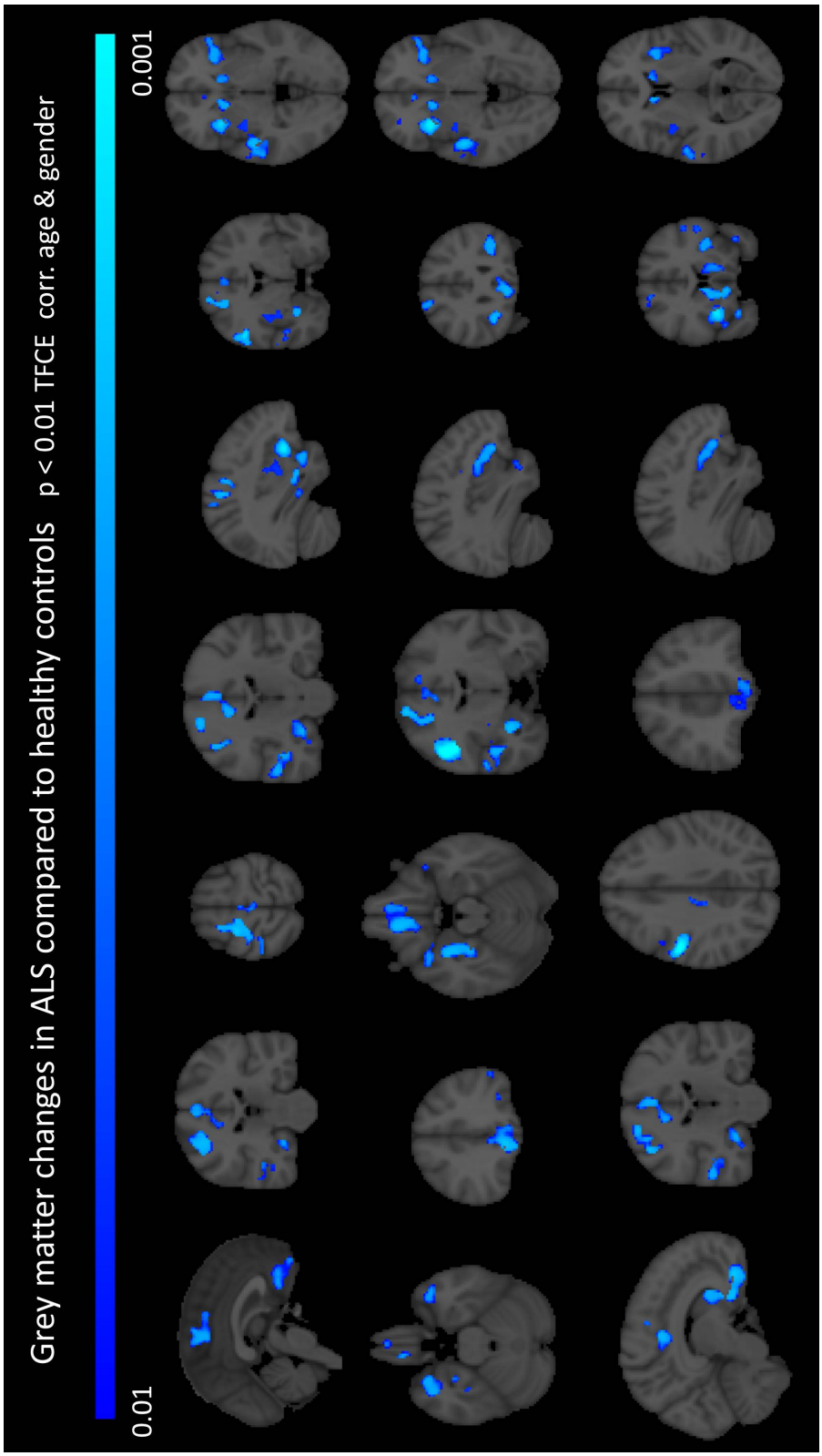


FIGURE 6*: Grey matter pathology in ALS at p < 0.01 TFCE FWE*

White matter alterations in ALS

Significant FA reductions were observed in ALS compared to healthy controls (**Figure 7**) at p < 0.01 TFCE FWE. The anatomical locations of white matter pathology included the corpus callosum, brain stem, superior longitudinal fasciculi, inferior longitudinal fasciculi, corticospinal tracts, forceps minor, forceps major, in addition to white matter tracts in the cerebellum.

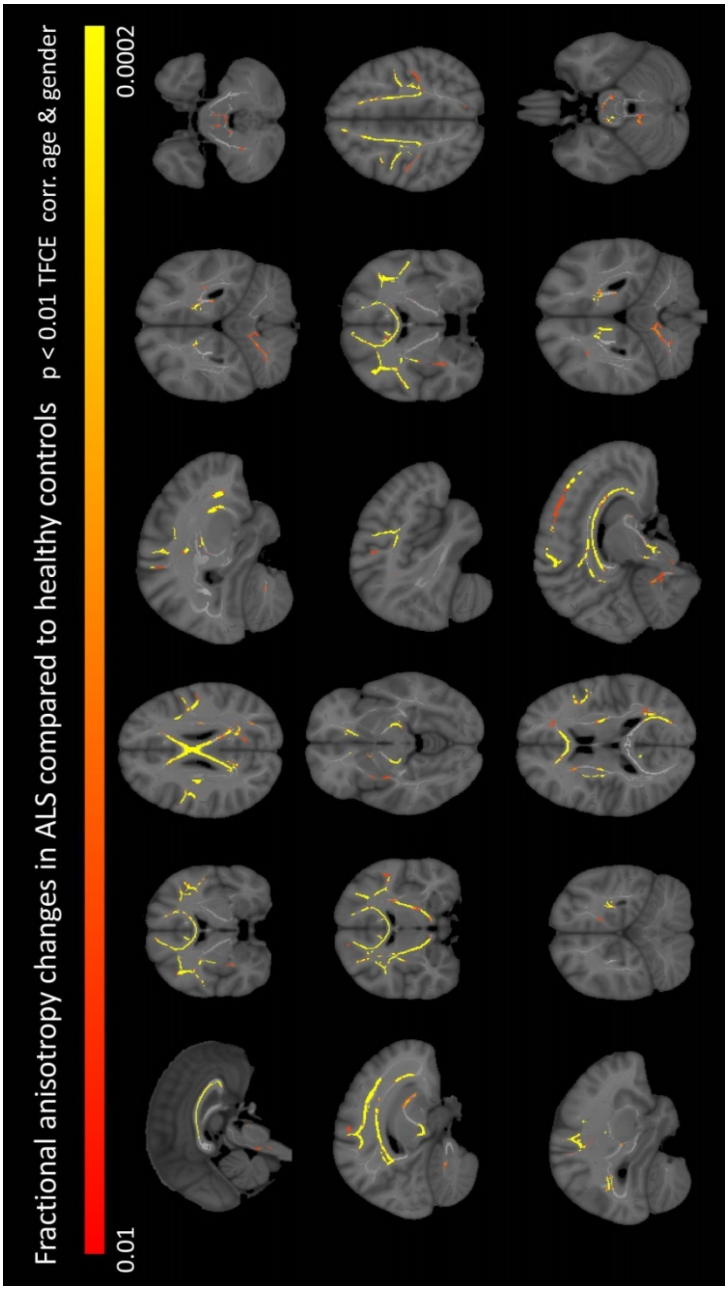


FIGURE 7: *Patterns of FA reductions in ALS compared to controls at p < 0.01 TFCE FWE*

AD alterations are less widespread than FA changes **(Figure 8)**, but significant AD increase was detected in the corpus callosum, corticospinal tracts, forceps minor, fornix, right inferior longitudinal fasciculus, and right uncinate fasciculus.

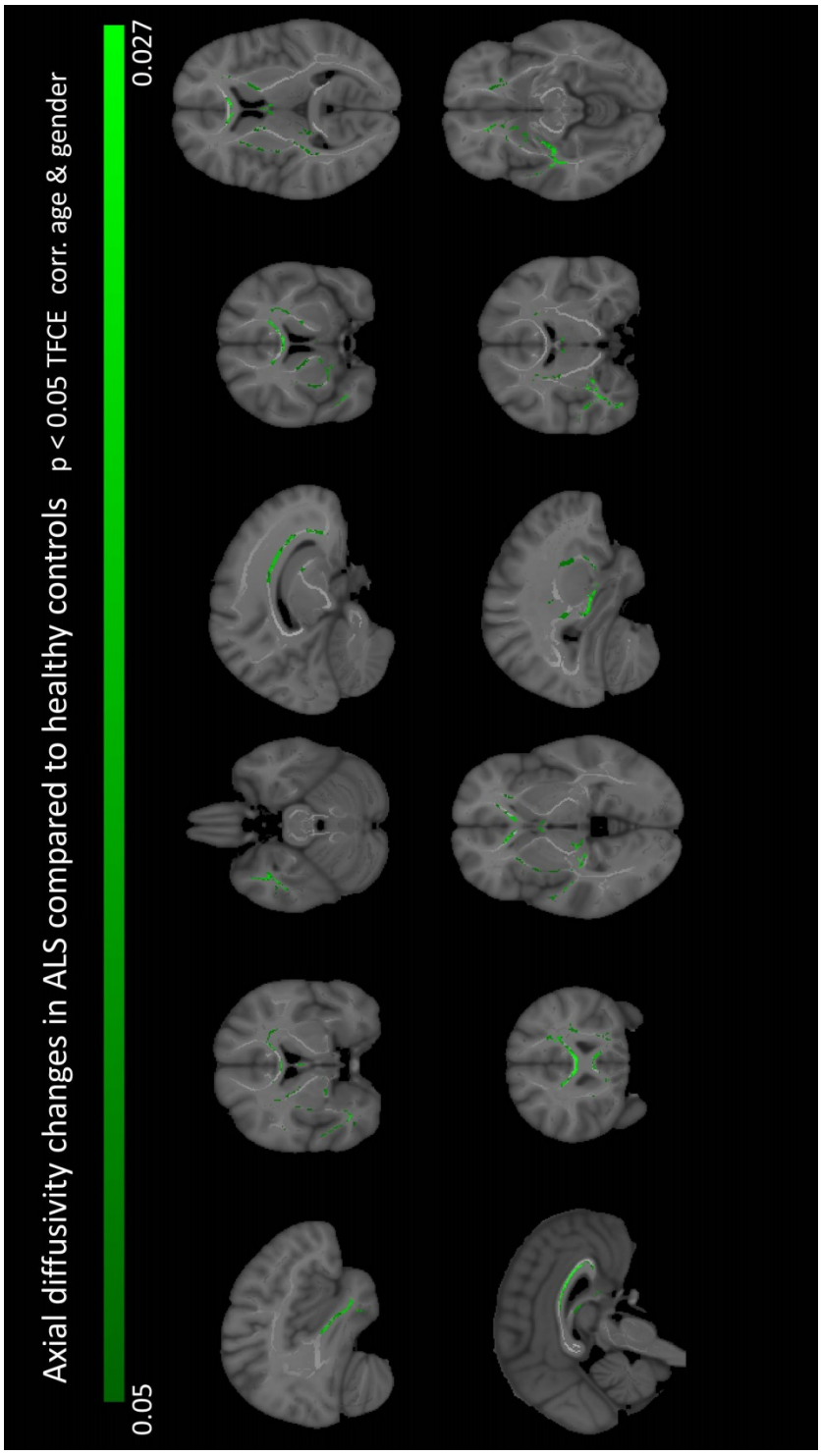


FIGURE 8: *Increased AD in compared to controls at p < 0.05 TFCE FWE*

Widespread RD increase was detected in the corticospinal tracts, corpus callosum, superior longitudinal fasciculi, right inferior longitudinal fasciculus, uncinate fasciculi, forceps minor and white matter tracts in the cerebellum **(Figure 9).**

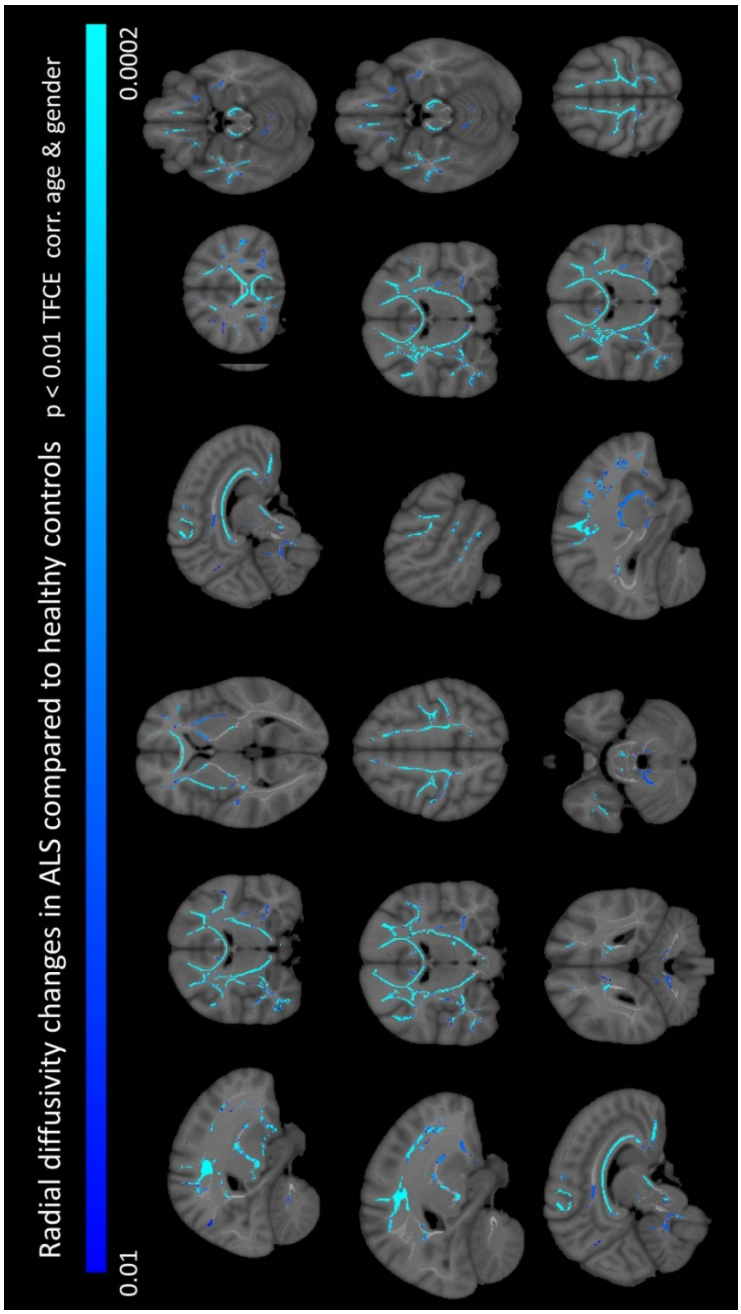


FIGURE 9: *RD changes in ALS compared to controls at p < 0.01 TFCE FWE*

Region of interest grey and white matter findings

ROI analysis findings

Grey matter (GM) volumes of the primary motor cortex (PMC), post central gyrus cerebellum, frontal lobe, temporal lobe, occipital lobe and parietal lobe were retrieved. Average fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) values of the corpus callosum (CC), corticospinal tract (CST), cerebellum, frontal lobe, temporal lobe, occipital lobe and parietal lobe were also retrieved.

Following the retrieval of grey and white matter metrics from the above ROIs, these were plotted in boxplots for explorative purposes before further statistics were carried out. The line in the middle of boxplot represents the median, the bottom and top box boundaries depict the first and third quartiles. The whiskers show the minimum and maximum values, with the exceptions of outliers shone as circles. While these raw values were uncorrected for demographic factors the boxplots revealed strikingly divergent imaging profiles in the pre-defined ROIs. **Figure 10** shows the distribution of GM metrics and **Figures 11, 12 and 13** show the boxplots of group-wise white matter metrics.

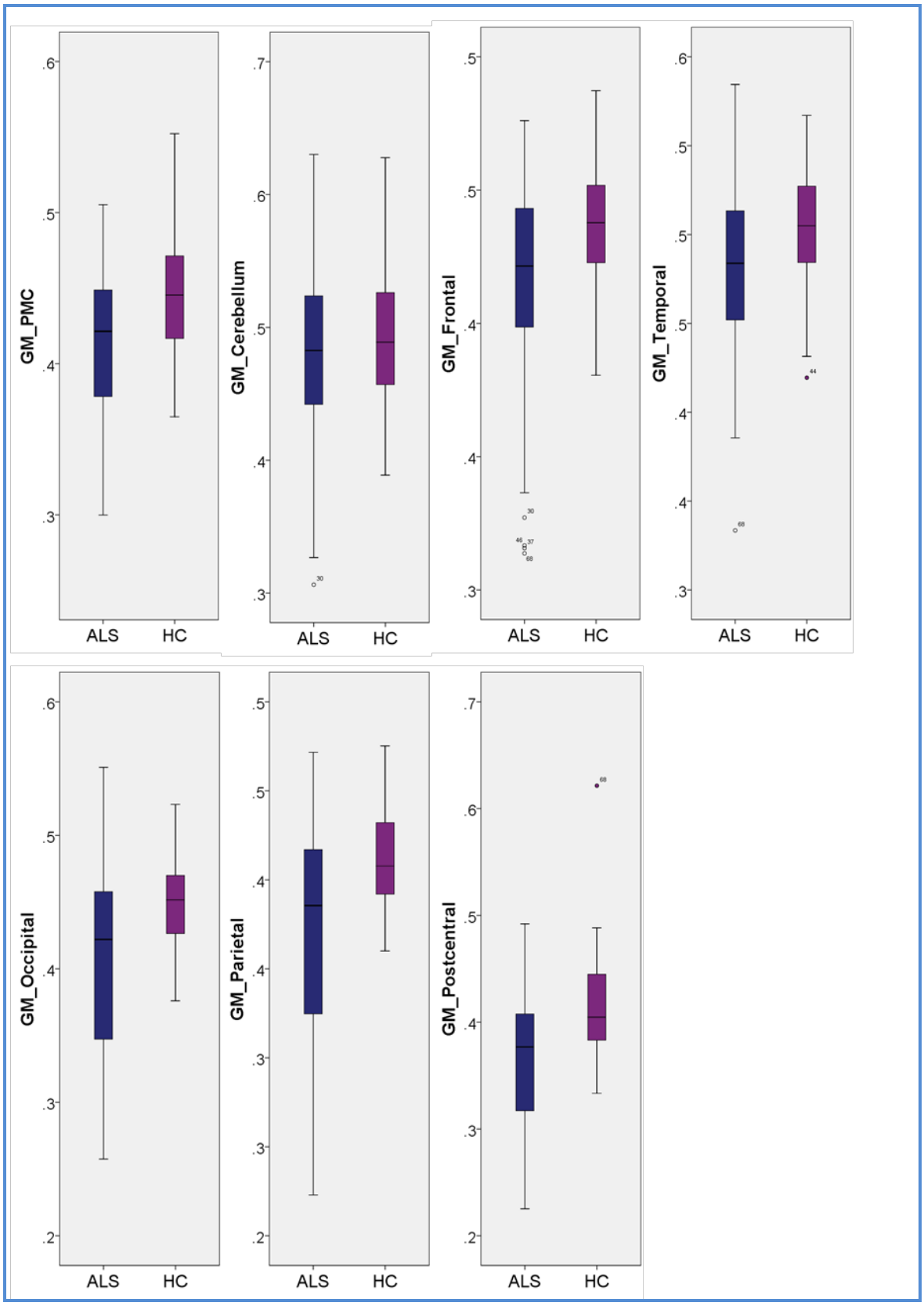


FIGURE 10: *The grey matter profile of ALS patients and healthy controls – raw ROI data*

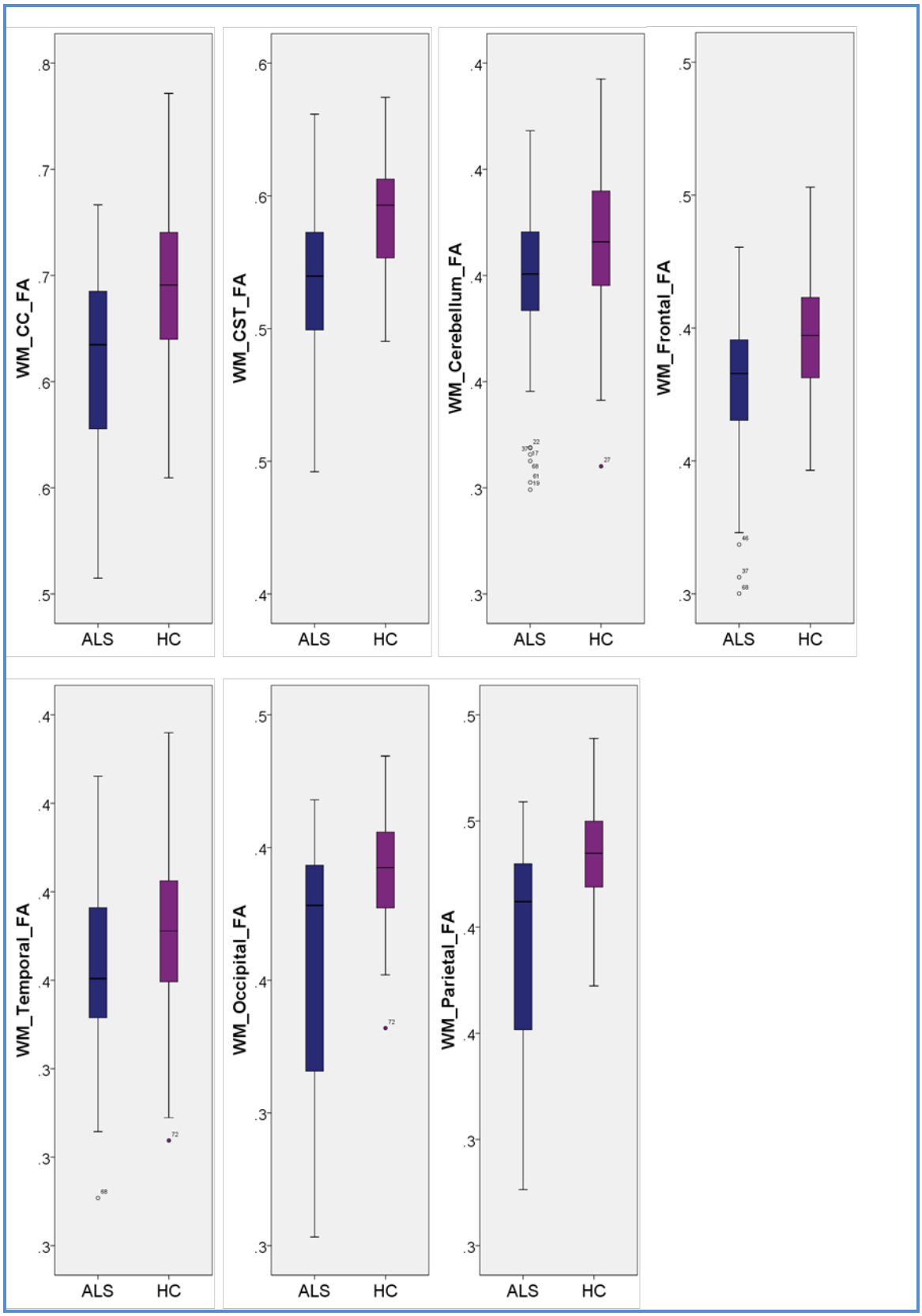


FIGURE 11: *The fractional anisotropy (FA) in ALS patients and healthy controls– raw ROI data*

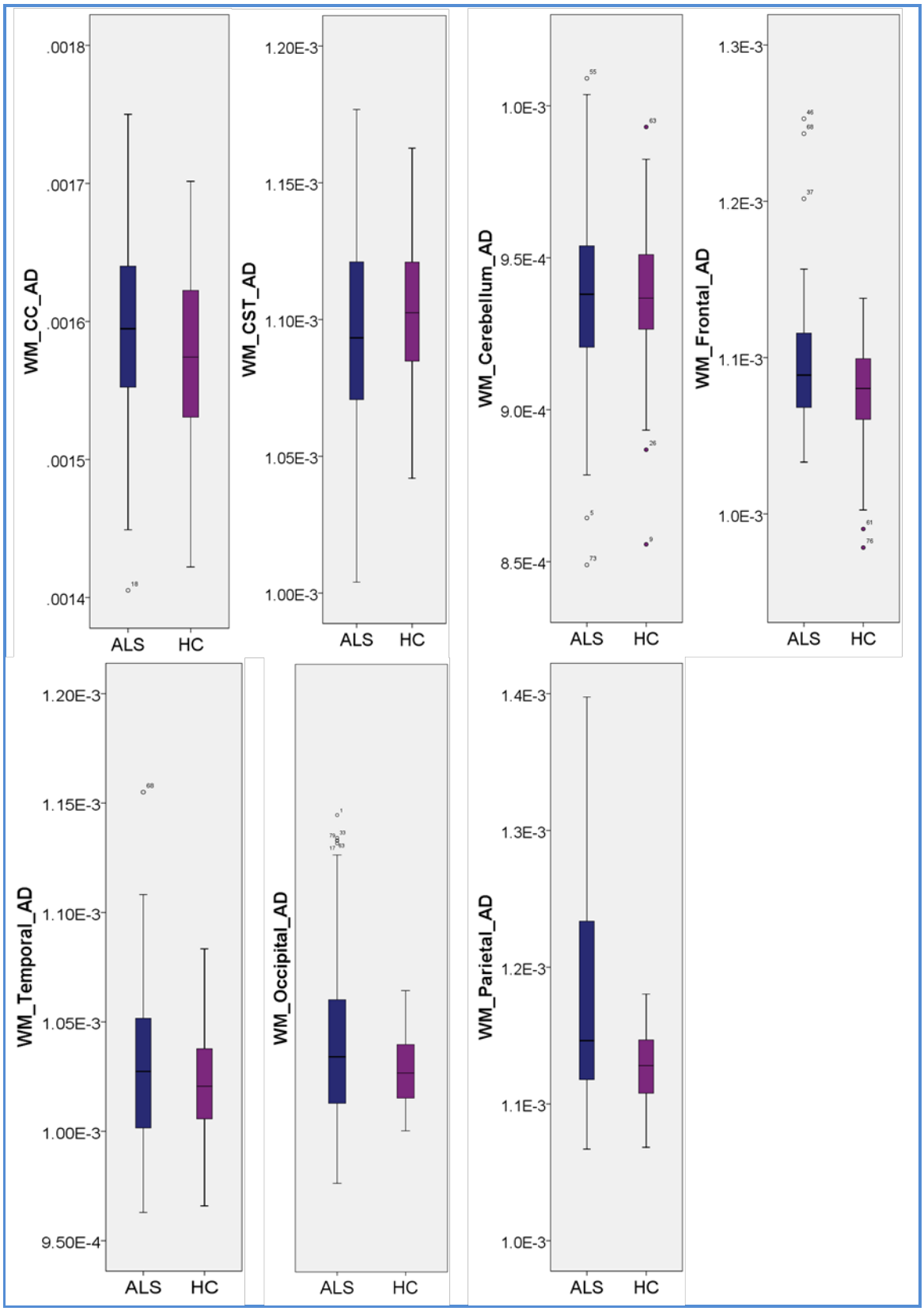


FIGURE 12 *The axial diffusivity (AD) profile of ALS patients and healthy controls – raw ROI data*

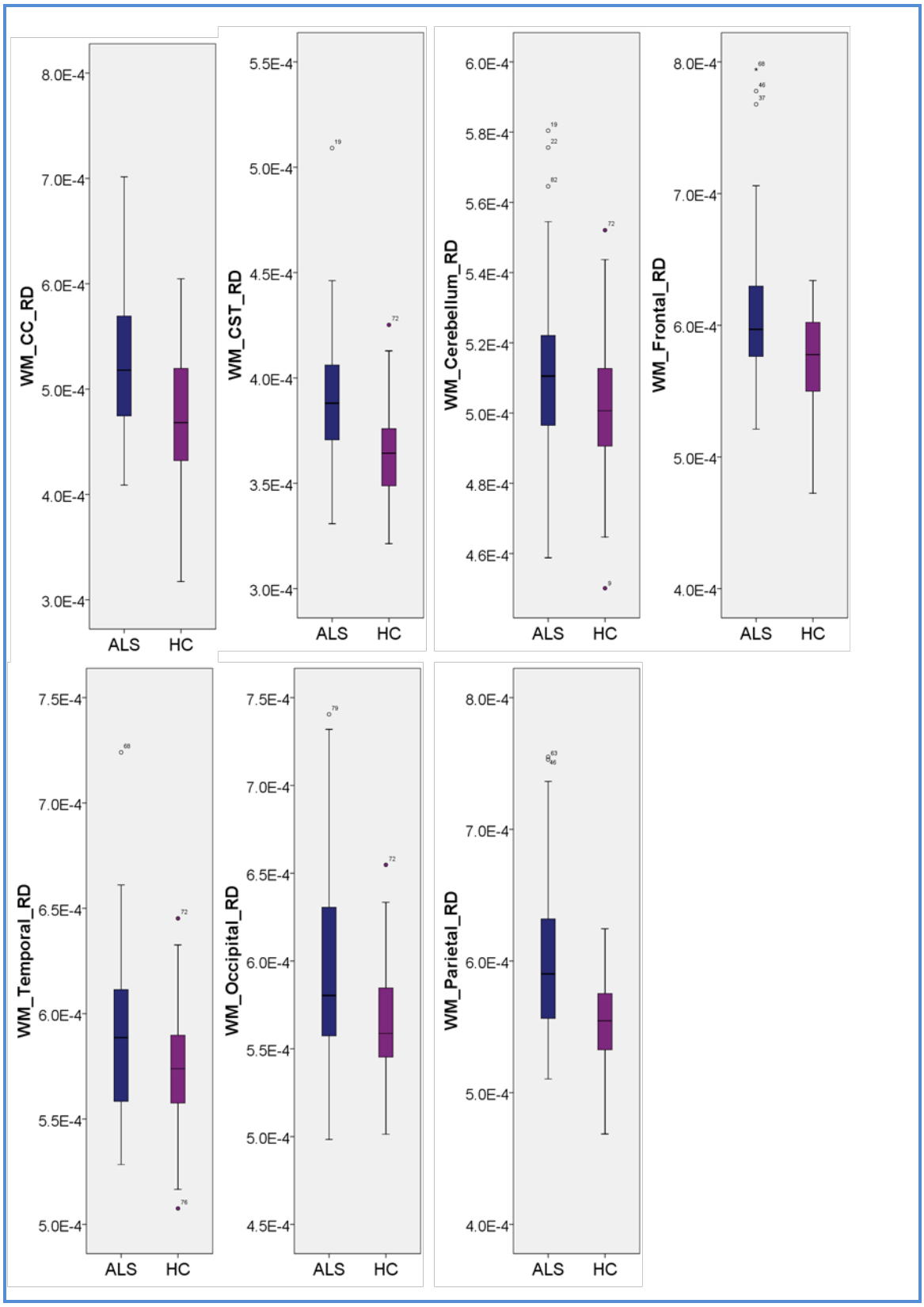


FIGURE 13: *The raw radial diffusivity (RD) in ALS patients and health controls – raw ROI data*

Analyses of covariance

As described in the methods section, ANCOVAs were preformed correcting for age and gender to compare the imaging profile of ALS patients and controls in the above ROIs. Grey matter volumes were very significantly reduced in ALS patients in comparison to healthy controls in the primary motor cortex (p = 0.000021), post central gyrus (p =0.000001), frontal lobe (p = 0.000245), temporal lobe (p = 0.001131), occipital lobe (p = 0.000011) and parietal lobe (p = 0.000002). No significant cerebellar grey matter atrophy was detected in ALS compared to controls (p = 0.613).

FA values were significantly reduced in ALS patients compared to healthy controls in all ROIs; the corpus callosum (p = 0.000001), corticospinal tracts (p = 1.65E-10), cerebellum (p = 0.012162), frontal lobe (p = 4.36E-7), temporal lobe (p = 0.010008), occipital lobe (p = 4.28E-7) and parietal lobe (p = 8.08E-9).

A trend of increased AD was detected in ALS compared to controls in the corticospinal tract (p = 0.088763), and significantly increased AD was captured in the frontal lobe (p = 0.009382) and parietal lobe (p = 0.000007).

RD values were significantly increased in all ROIs; the corpus callosum (p = 6.59E-7), the CST (p = 8.80E-10), the cerebellum (p = 0.017268), the frontal lobe (p =0.000006), the temporal lobe (p = 0.006930), the occipital lobe (p = 0.000125) and the parietal lobe (5.80E-8) **(Table 13).**

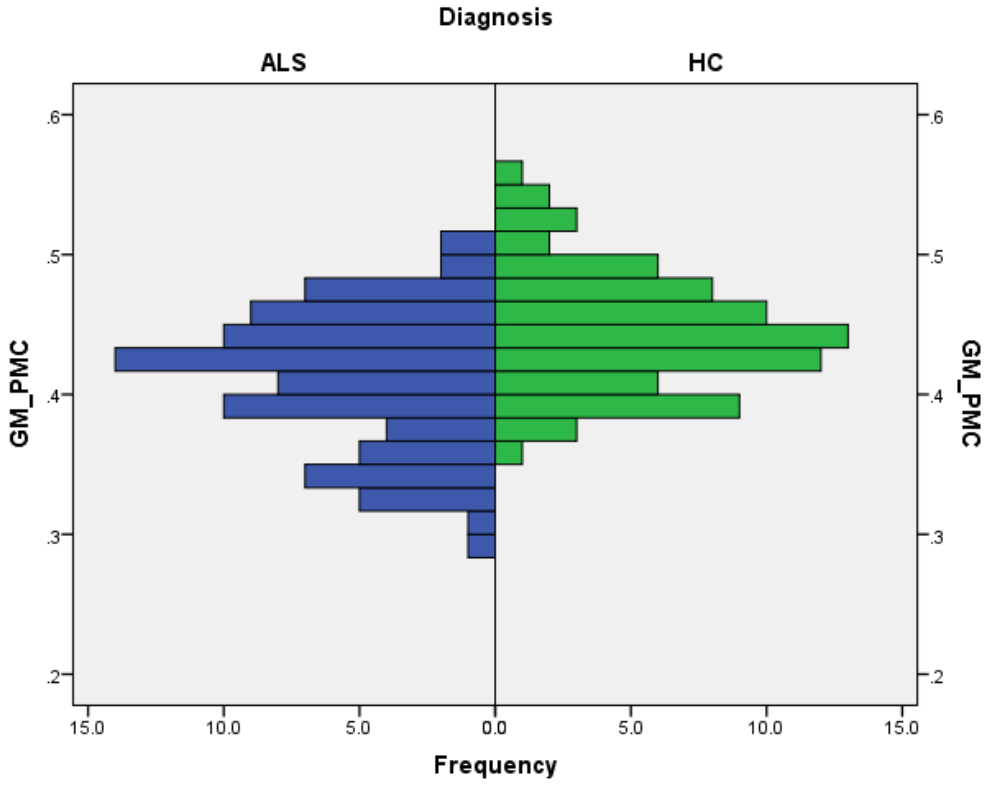
Estimated marginal values for grey matter volume, fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD)				
Region of interest	**Study group**	**Estimated marginal values adjusted for age and gender**	**Standard error**	**ANCOVA Sig. (p)** **at least 6 decimal places**
GM Primary Motor Cortex	Healthy Controls	0.4422195	0.0045048	**0.000021**
	ALS patients	0.4147913	0.0042560	
GM Cerebellum	Healthy Controls	0.4893127	0.0059395	0.612501
	ALS patients	0.4851342	0.0056114	
GM Frontal Lobe	Healthy Controls	0.4349936	0.0032774	**0.000245**
	ALS patients	0.4179413	0.0030964	
GM Temporal Lobe	Healthy Controls	0.5018114	0.0043465	**0.001131**
	ALS patients	0.4818276	0.0041065	
GM Occipital Lobe	Healthy Controls	0.4483800	0.0059122	**0.000011**
	ALS patients	0.4110939	0.0055857	
GM Parietal Lobe	Healthy Controls	0.4082496	0.0053922	**0.000002**
	ALS patients	0.3715999	0.0050944	
GM Post Central Gyrus	Healthy Controls	0.4080003	0.0059848	**0.000001**
	ALS patients	0.3662299	0.0056543	
FA Corpus Callosum	Healthy Controls	0.6431970	0.0043192	**0.000001**
	ALS patients	0.6128120	0.0040807	
FA Corticospinal Tract	Healthy Controls	0.5420158	0.0024539	**1.65E-10**
	ALS patients	0.5187439	0.0023183	
FA Cerebellum	Healthy Controls	0.3852593	0.0015123	**0.012162**
	ALS patients	0.3799415	0.0014287	

FA Frontal Lobe	Healthy Controls	0.3962573	0.0023068	**4.36E-7**
	ALS patients	0.3793933	0.0021794	
FA Temporal Lobe	Healthy Controls	0.3694501	0.0018283	**0.010008**
	ALS patients	0.3628424	0.0017274	
FA Occipital Lobe	Healthy Controls	0.3909170	0.0044174	**4.28E-7**
	ALS patients	0.3585976	0.0041735	
FA Parietal Lobe	Healthy Controls	0.4321117	0.0047531	**8.08E-9**
	ALS patients	0.3919435	0.0044906	
AD Corpus Callosum	Healthy Controls	0.0015772	0.0000068	0.115226
	ALS patients	0.0015921	0.0000064	
AD Corticospinal Tract	Healthy Controls	0.0011034	0.0000036	0.088763
	ALS patients	0.0010949	0.0000034	
AD Cerebellum	Healthy Controls	0.0009373	0.0000030	0.889846
	ALS patients	0.0009379	0.0000028	
AD Frontal	Healthy Controls	0.0010785	0.0000039	**0.009382**
	ALS patients	0.0010929	0.0000037	
AD Temporal	Healthy Controls	0.0010238	0.0000035	0.265727
	ALS patients	0.0010291	0.0000033	
AD Occipital	Healthy Controls	-0.0009488	0.0105964	0.234792
	ALS patients	0.0165697	0.0100112	
AD Parietal	Healthy Controls	0.0011288	0.0000083	**0.000007**
	ALS patients	0.0011827	0.0000079	
RD Corpus Callosum	Healthy Controls	0.0004747	0.0000068	**6.59E-7**
	ALS patients	0.0005235	0.0000064	
RD Corticospinal	Healthy Controls	0.0003655	0.0000026	**8.80E-10**

Tract				
	ALS patients	0.0003889	0.0000024	
RD Cerebellum	Healthy Controls	0.0005019	0.0000023	**0.017268**
	ALS patients	0.0005096	0.0000022	
RD Frontal	Healthy Controls	0.0005785	0.0000044	**0.000006**
	ALS patients	0.0006068	0.0000041	
RD Temporal	Healthy Controls	0.0005749	0.0000032	**0.006930**
	ALS patients	0.0005871	0.0000031	
RD Occipital	Healthy Controls	0.0005661	0.0000050	**0.000125**
	ALS patients	0.0005935	0.0000047	
RD Parietal	Healthy Controls	0.0005566	0.0000051	**5.80E-8**
	ALS patients	0.0005994	0.0000054	

TABLE 13: *Estimated marginal values for grey matter volume, fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD)*

Following the group-wise comparison of imaging metrics in the above ROIs, data were also plotted in histograms to visualise the distribution of imaging metrics across the study groups **(Figures 14, 15, 16 and 17).** In all ROIs the values were more homogenous in the control group than in the patient cohort.


Diagnosis
ALS
HC
GM_PMC
GM_PMC
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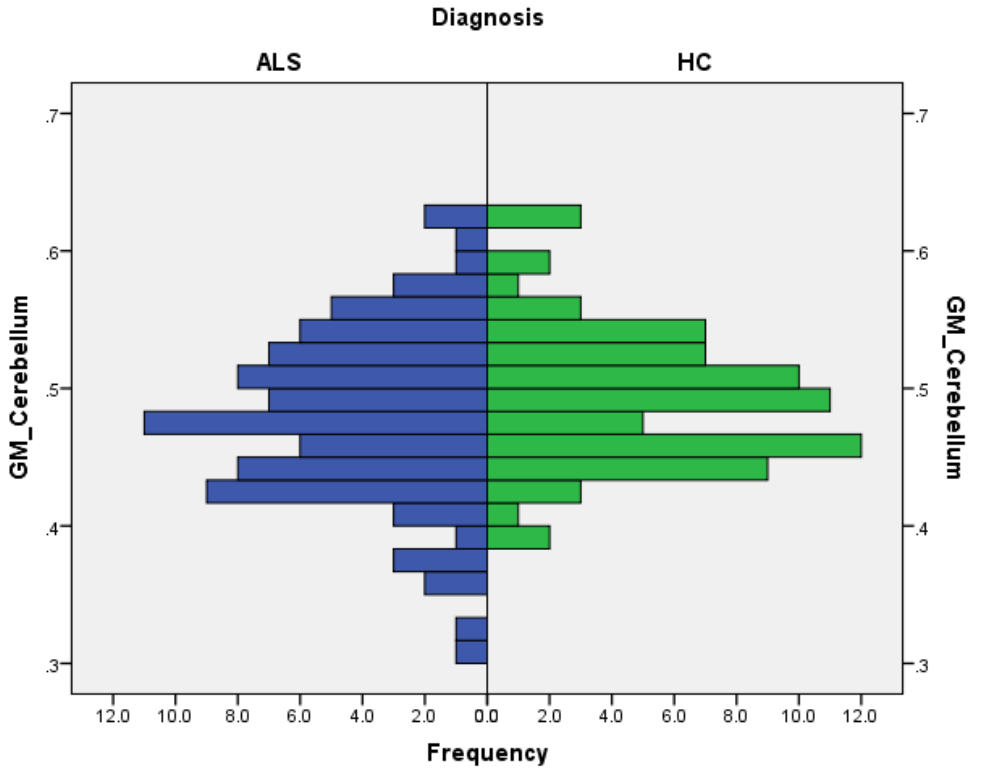
.5
.4
.3
.2
15.0
10.0
5.0
0.0
5.0
10.0
15.0
Frequency


Diagnosis
ALS
HC
GM_Cerebellum
GM_Cerebellum
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.6
.5
.4
.3
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0.0
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6.0
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10.0
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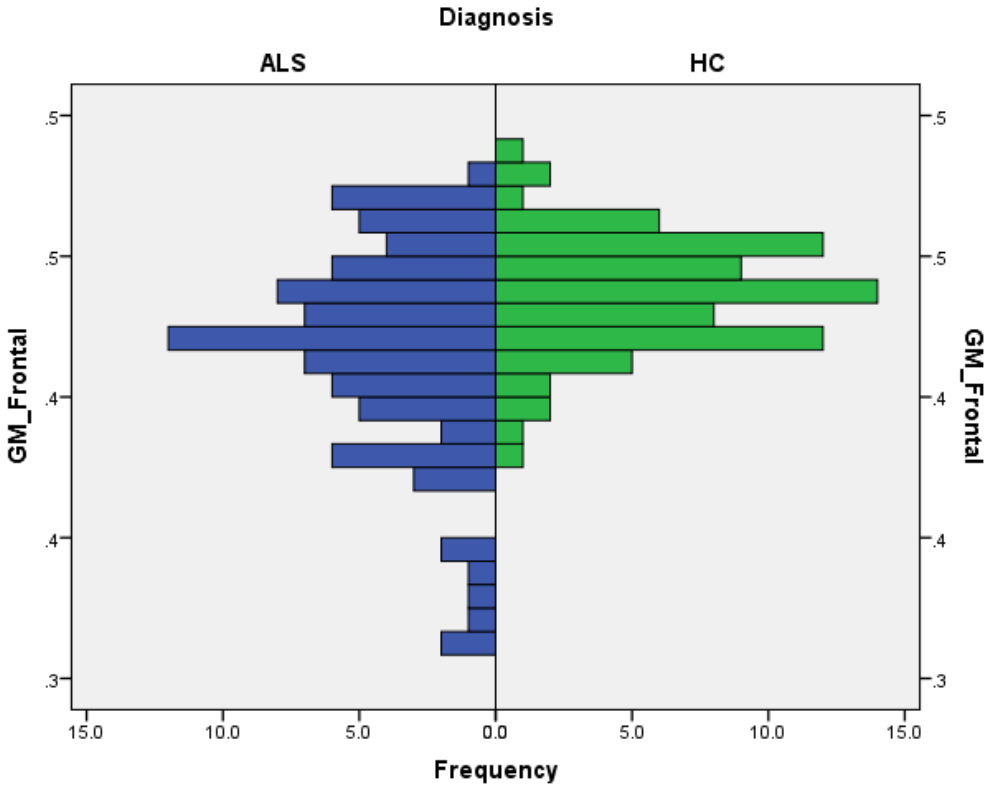
Frequency


Diagnosis
ALS
HC
GM_Frontal
GM_Frontal
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.4
.4
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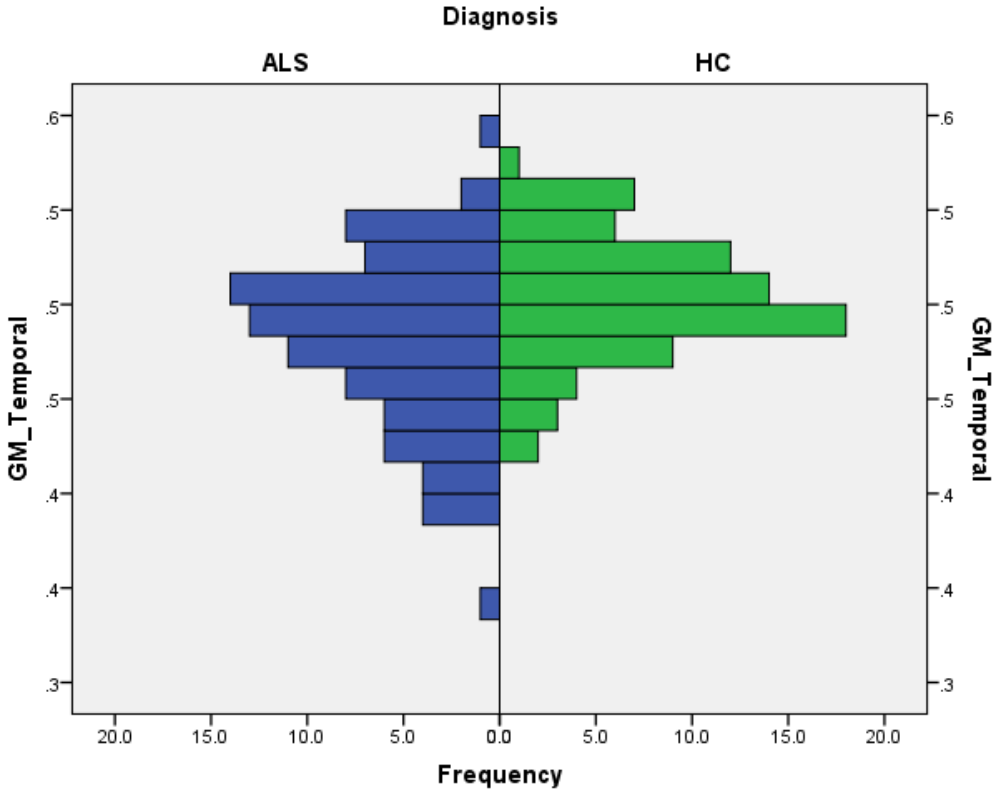
10.0
15.0
Frequency


Diagnosis
ALS
HC
GM_Temporal
GM_Temporal
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5.0
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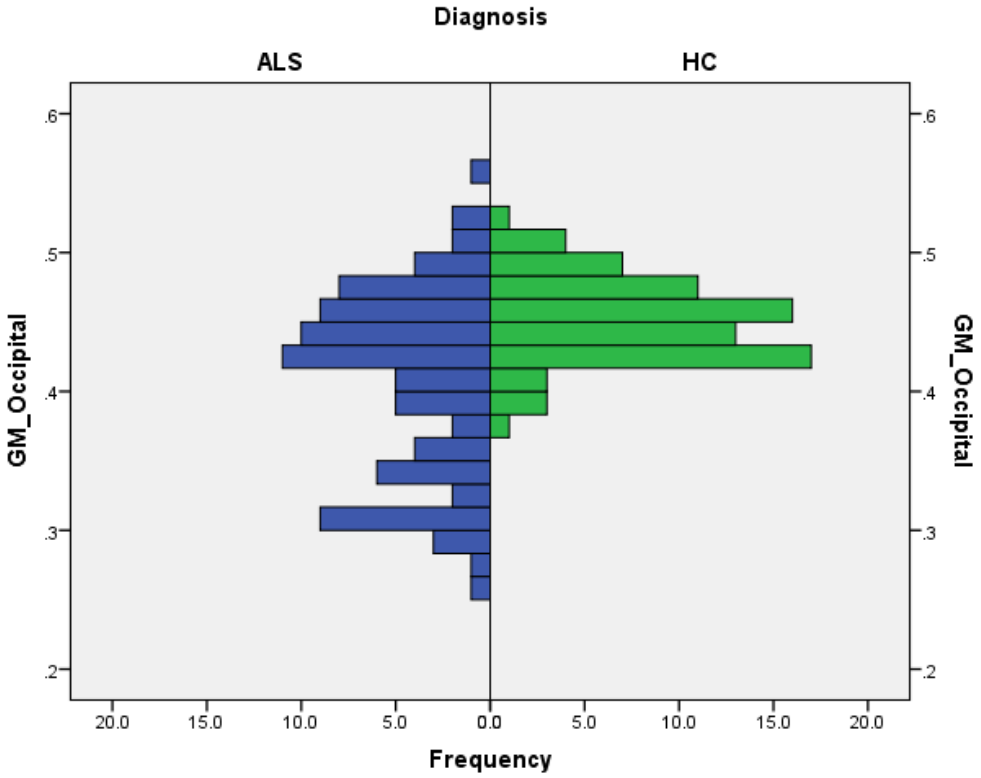
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
GM_Occipital
GM_Occipital
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.5
.4
.3
.2
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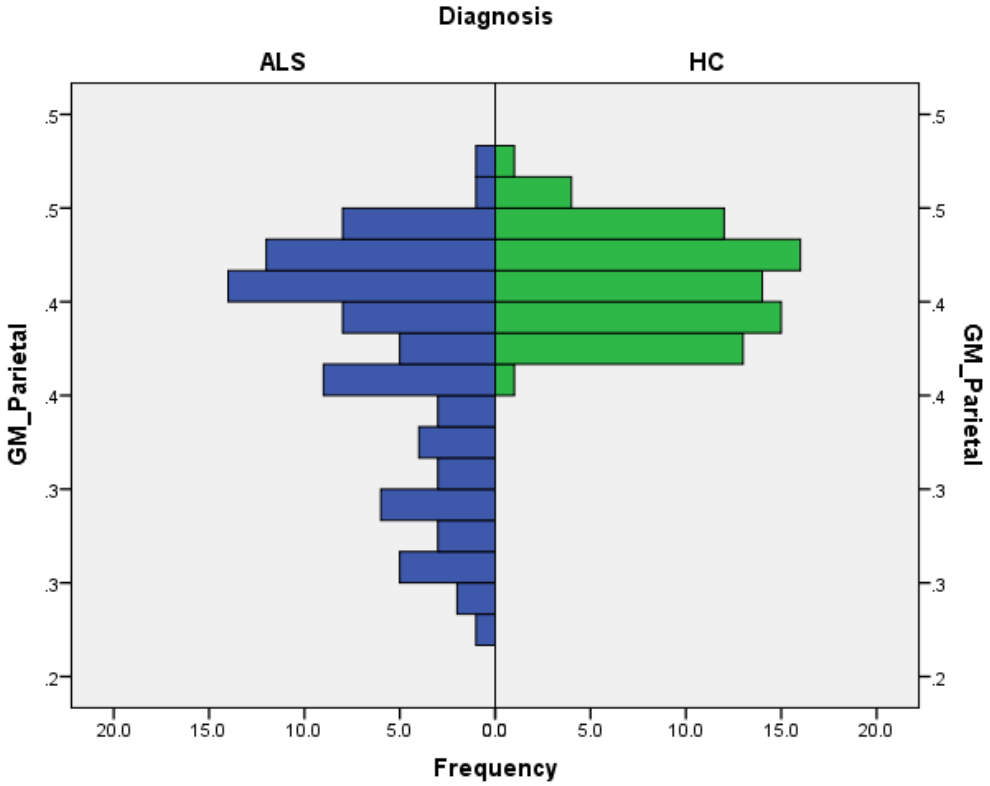
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
GM_Parietal
GM_Parietal
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.4
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5.0
10.0
15.0
20.0
Frequency

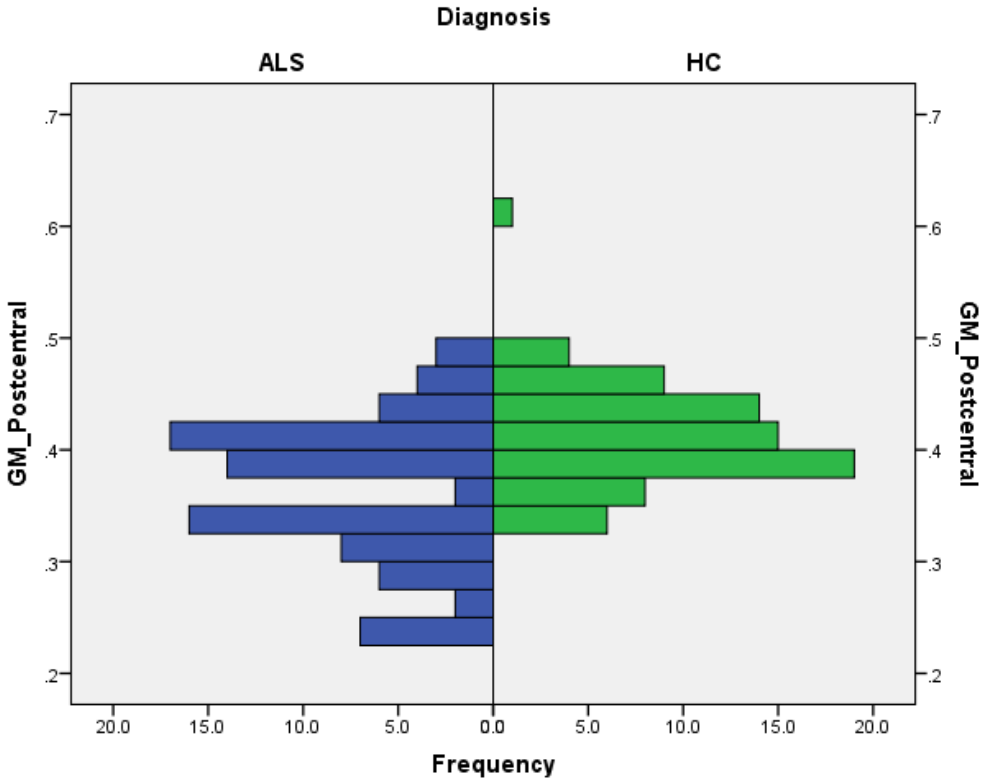
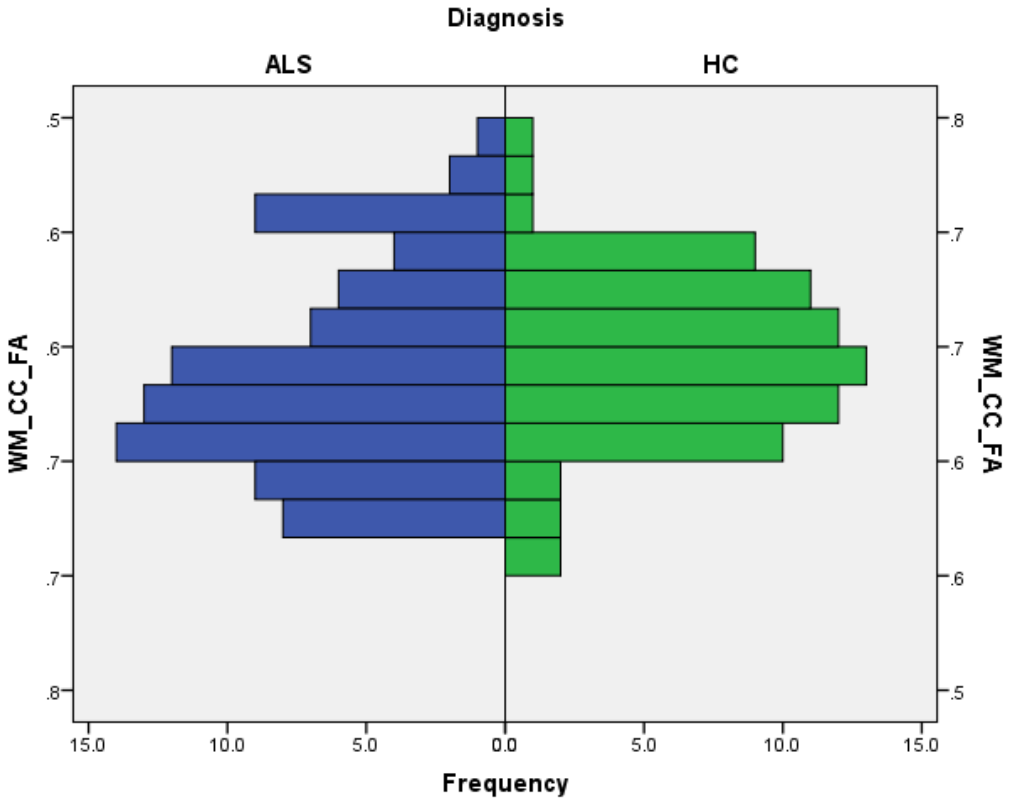


FIGURE 14: *Histograms of GM volumes in ALS patients and controls*


Diagnosis
ALS
HC
WM_CC_FA
WM_CC_FA
.5
.6
.6
.7
.8
.8
.7
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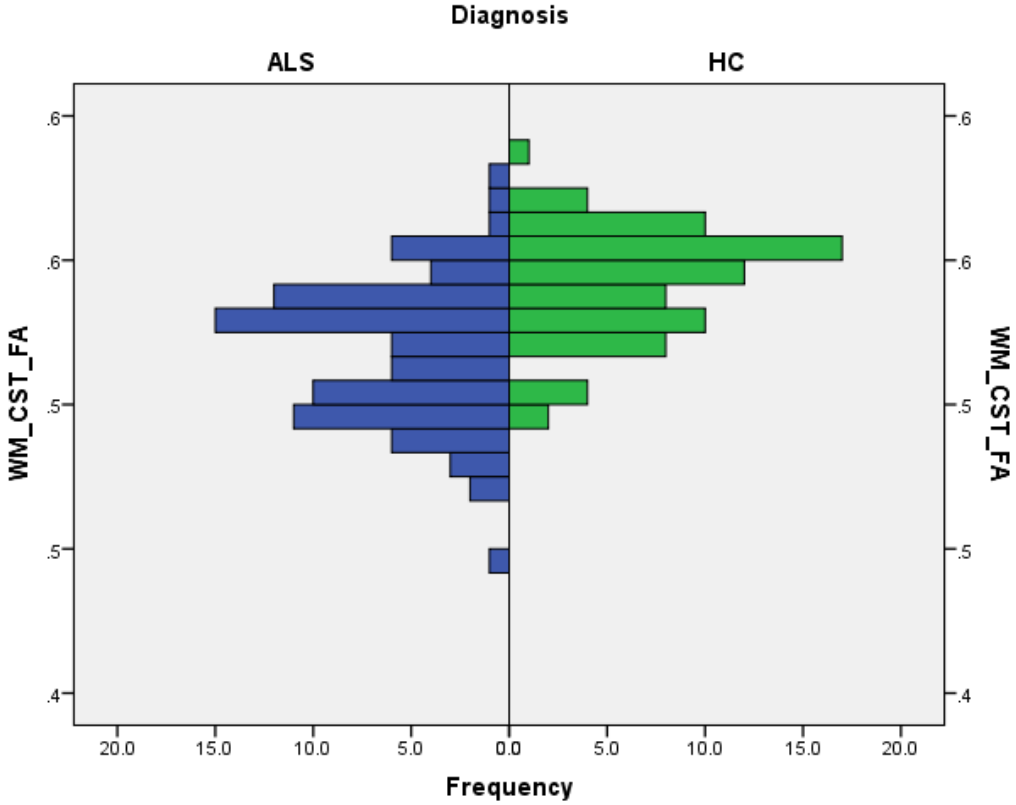
.6
.6
.5
15.0
10.0
5.0
0.0
5.0
10.0
15.0
Frequency


Diagnosis
ALS
HC
WM_CST_FA
WM_CST_FA
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.6
.6
.6
.5
.5
.5
.5
.4
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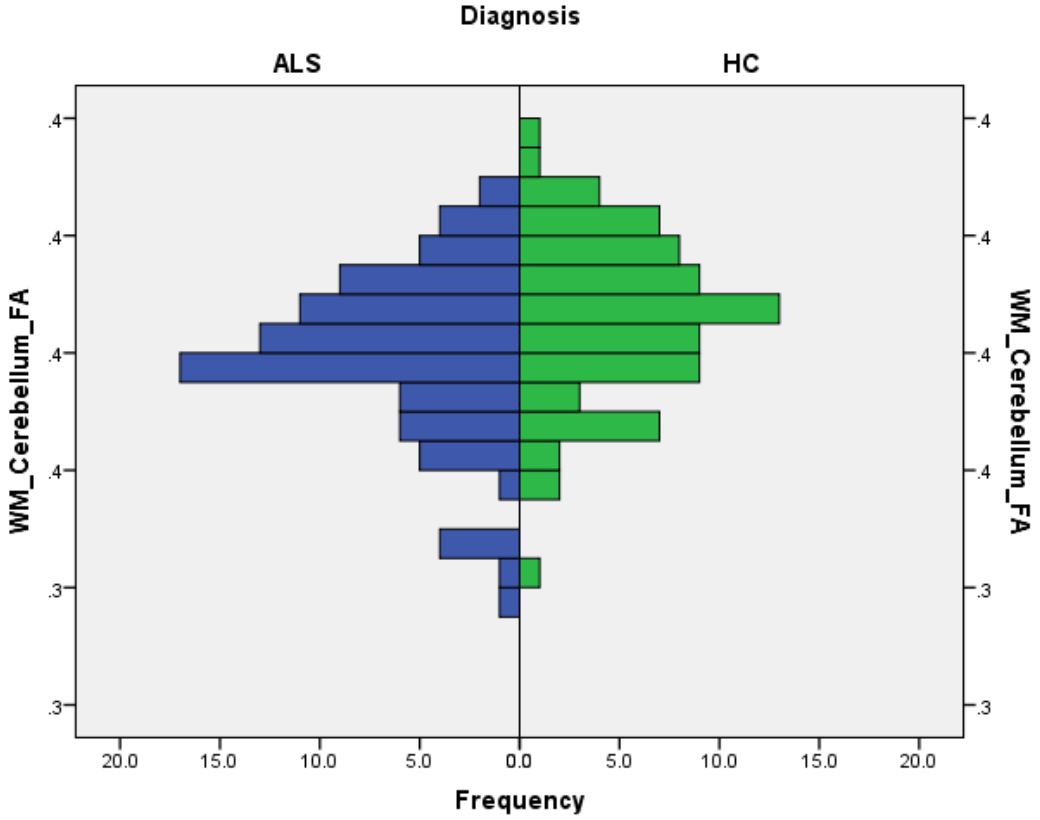
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Cerebellum_FA
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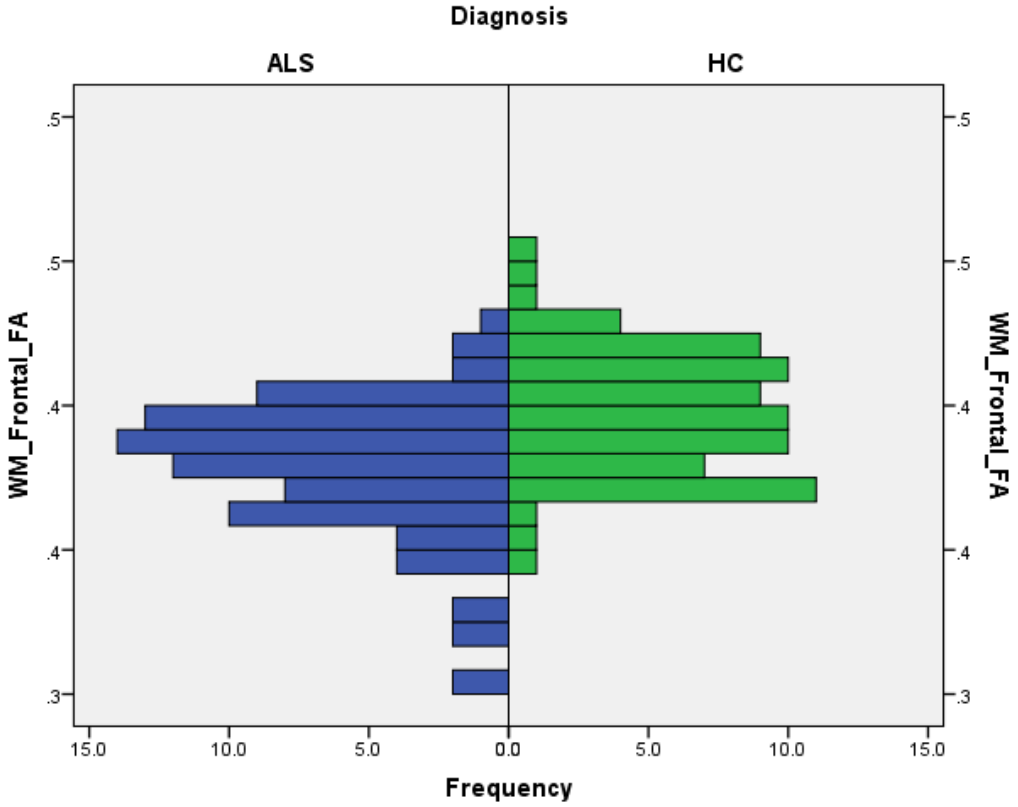
.3
20.0
15.0
10.0
5.0
0.0
Frequency


Diagnosis
ALS
HC
WM_Frontal_FA
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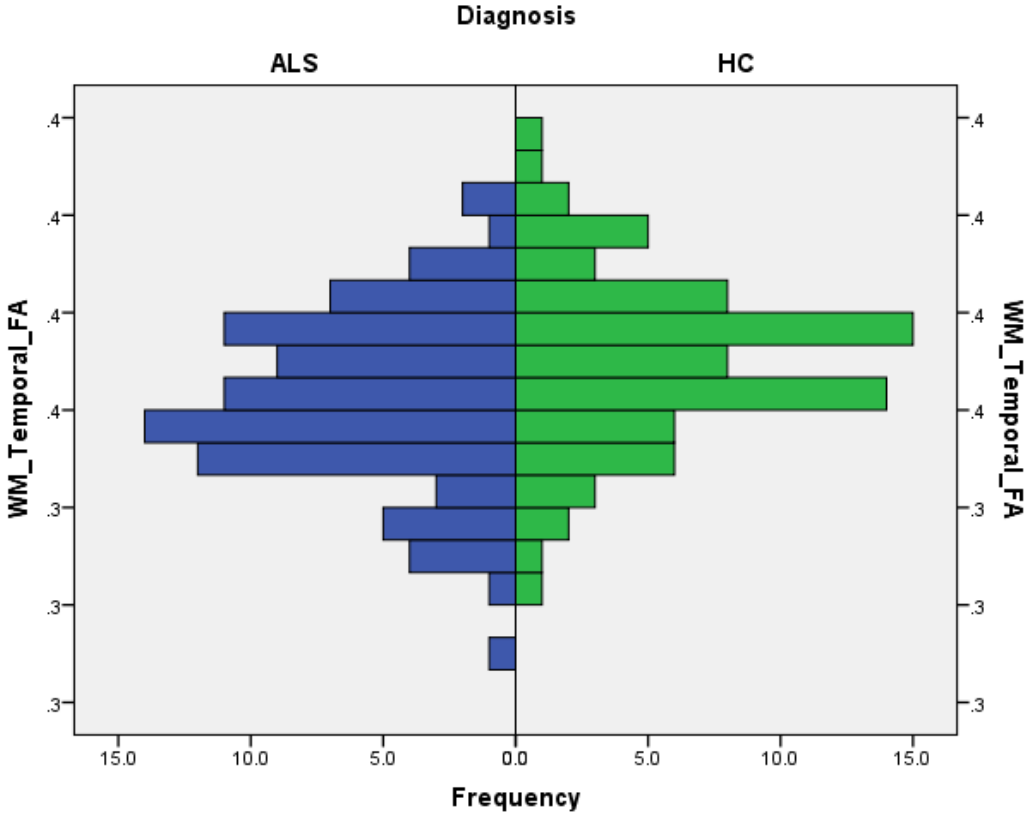
.4
.3
15.0
10.0
5.0
0.0
Frequency


Diagnosis
ALS
HC
WM_Temporal_FA
WM_Temporal_FA
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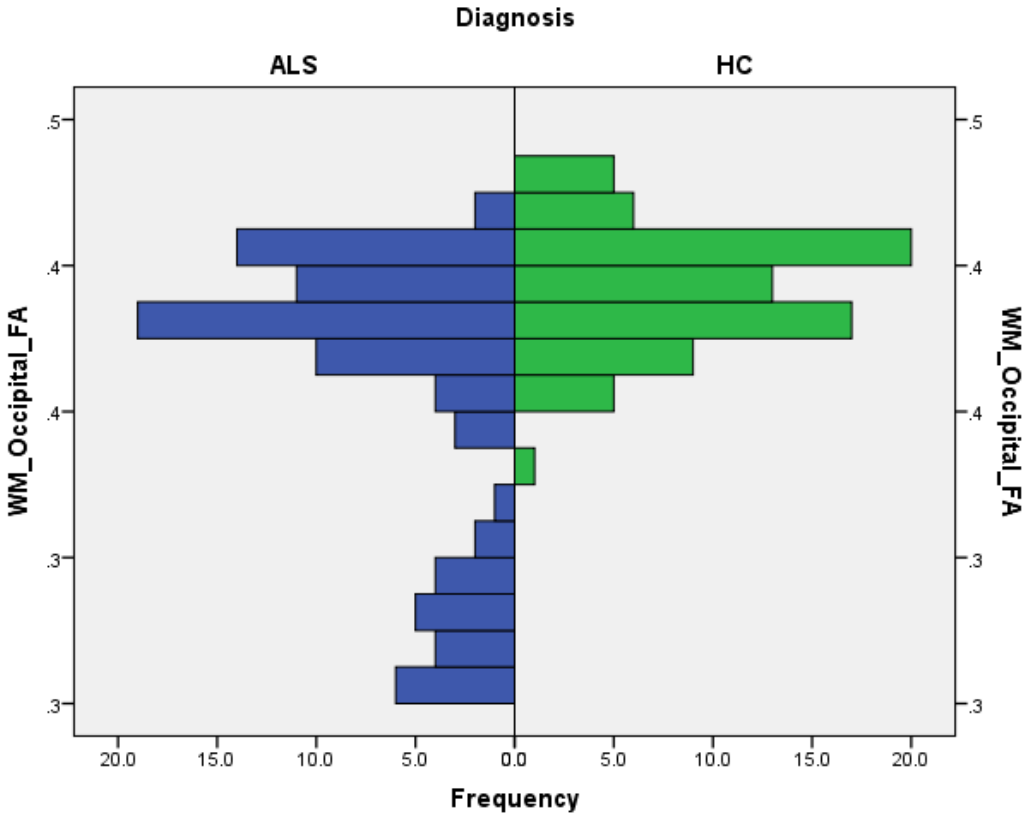
.4
.4
.4
.3
.3
.3
15.0
10.0
5.0
0.0
5.0
10.0
15.0
Frequency


Diagnosis
ALS
HC
WM_Occipital_FA
WM_Occipital_FA
.5
.4
.4
.3
.3
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency

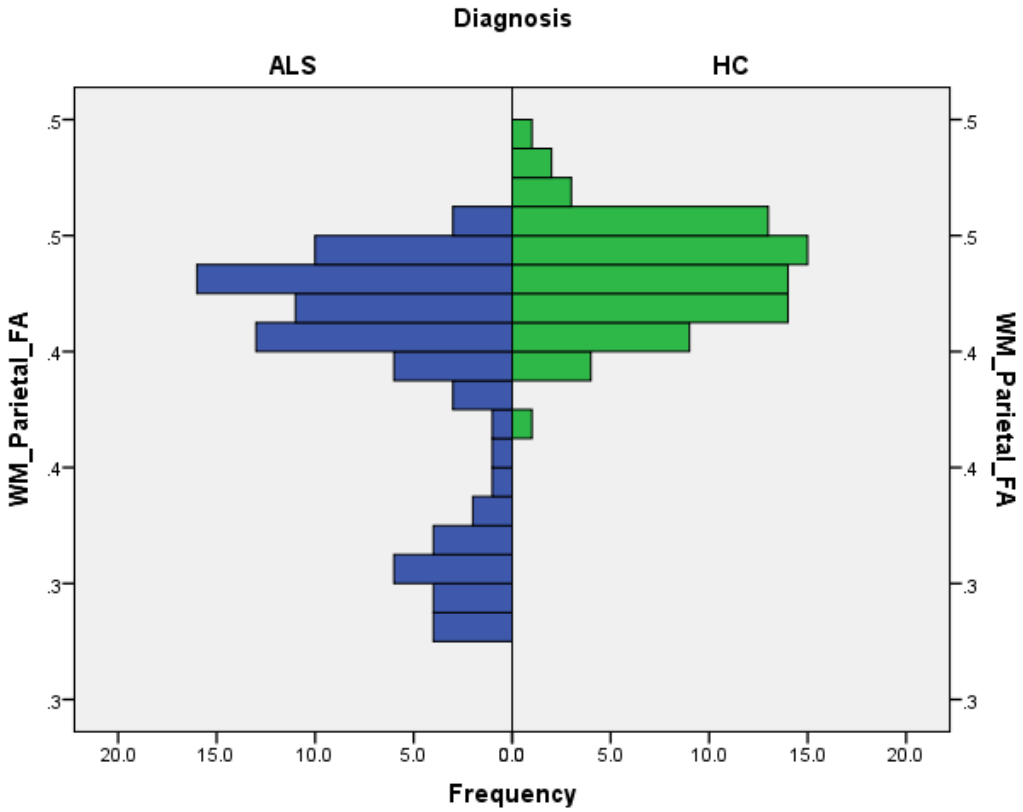
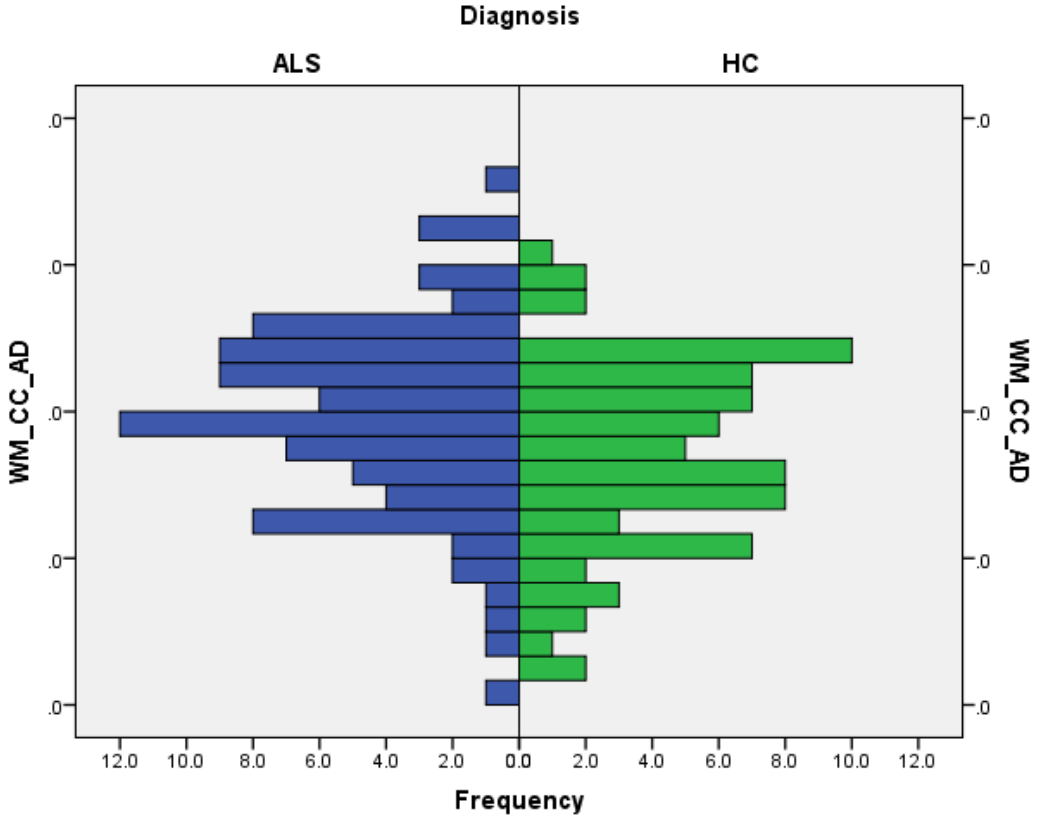


FIGURE 15*: Histograms of fractional anisotropy (FA) in ALS patients and healthy controls*


Diagnosis
ALS
HC
WM_CC_AD
WM_CC_AD
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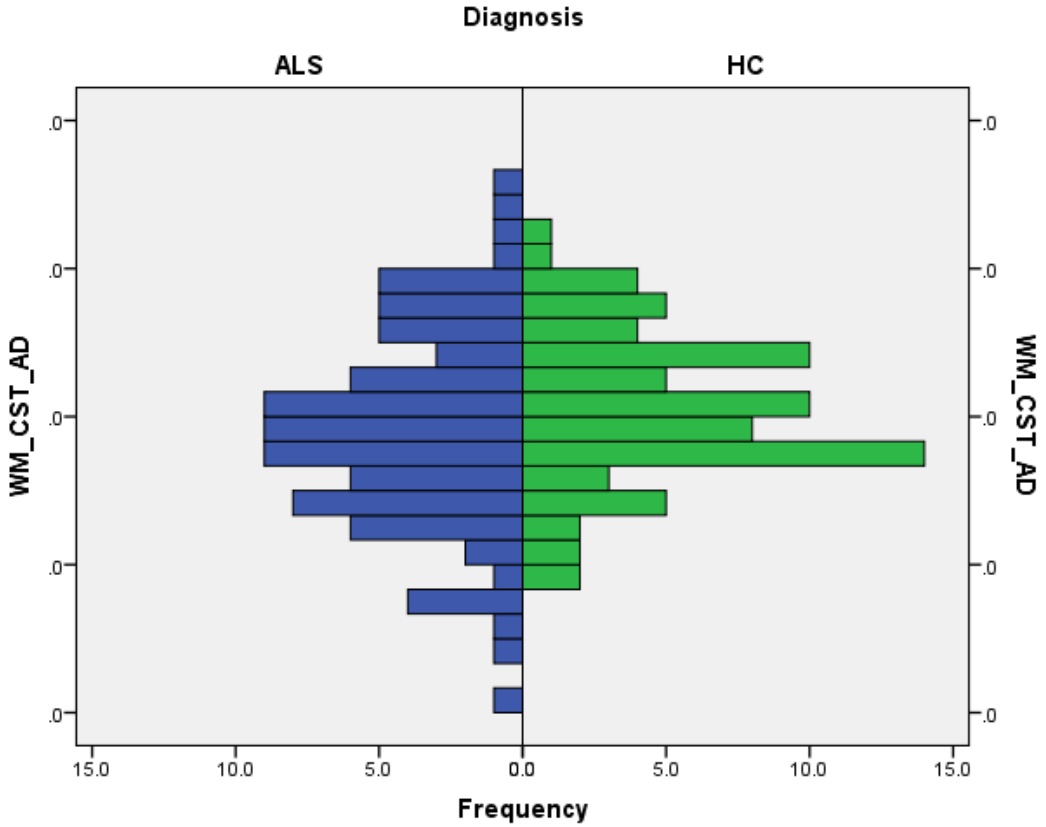
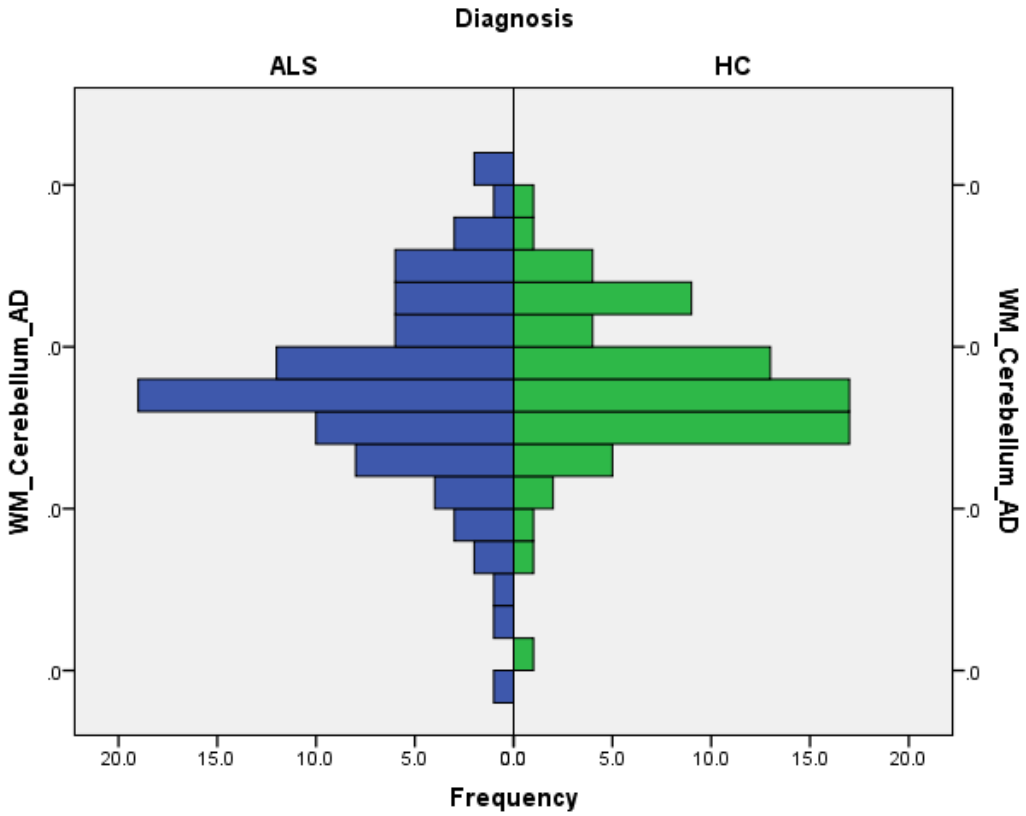
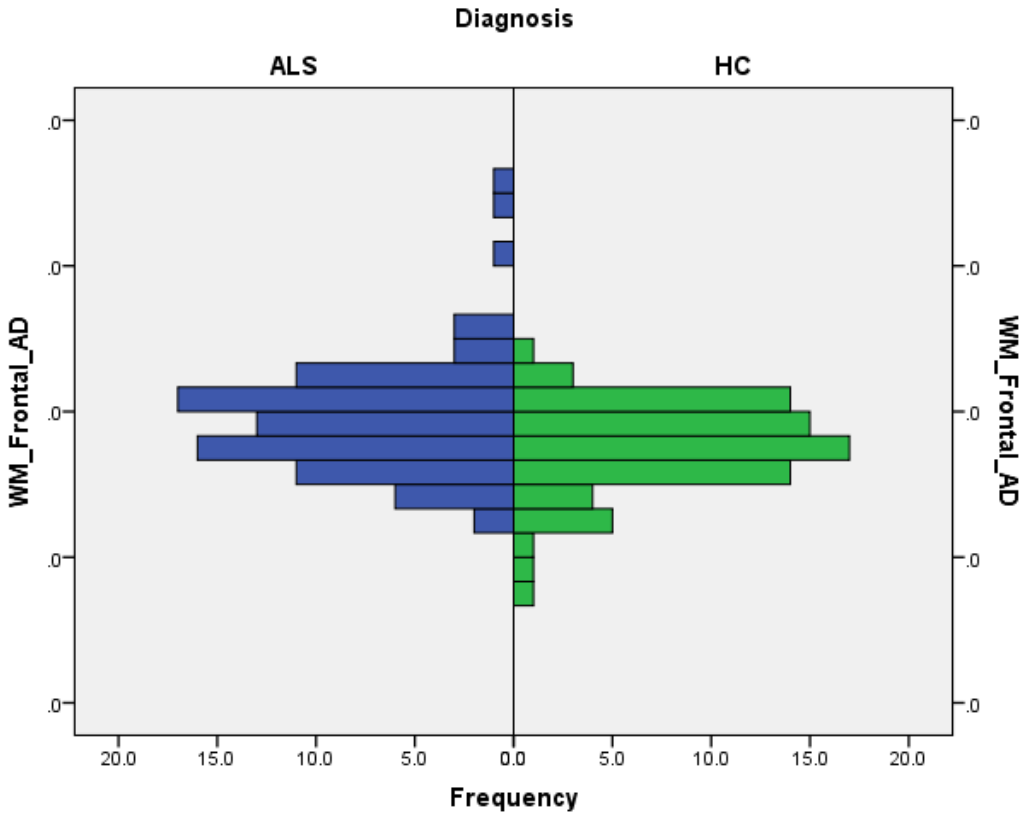
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Frequency


Diagnosis
ALS
HC
WM_CST_AD
WM_CST_AD
.0
15.0
10.0
5.0
0.0
Frequency


Diagnosis
ALS
HC
WM_Cerebellum_AD
WM_Cerebellum_AD
.0
.0
.0
.0
.0
.0
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Frontal_AD
WM_Frontal_AD
.0
.0
.0
.0
.0
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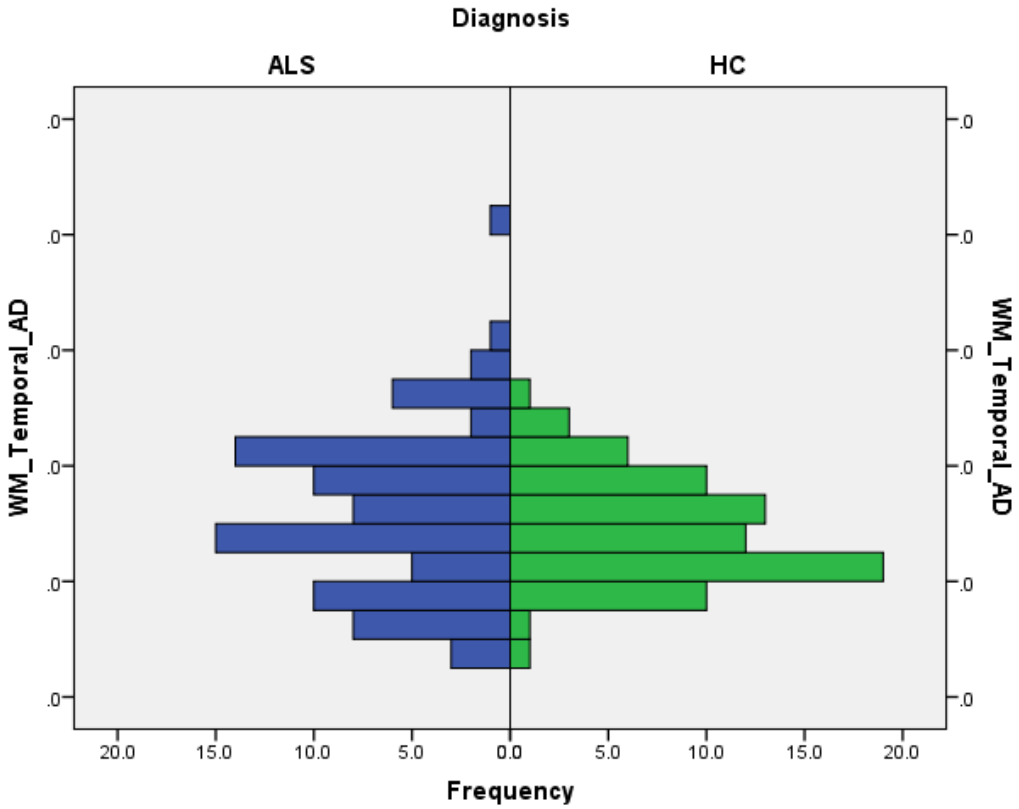
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Temporal_AD
WM_Temporal_AD
.0
.0
.0
.0
.0
.0
.0
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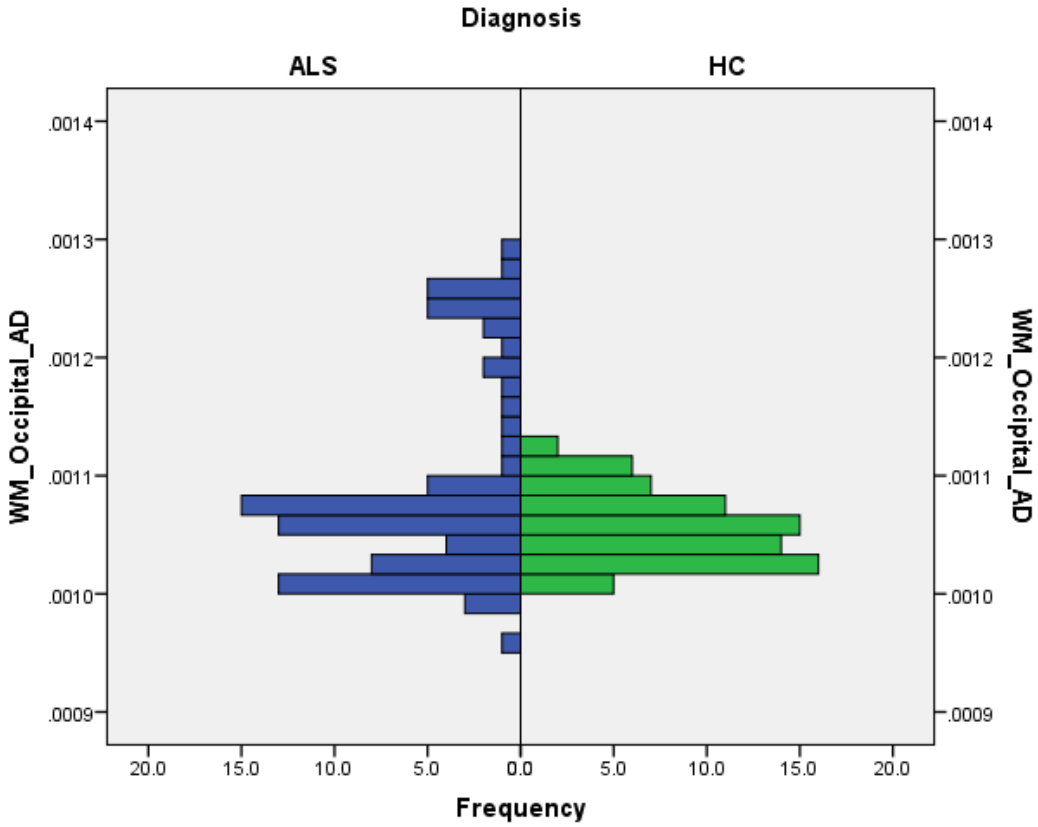
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Occipital_AD
WM_Occipital_AD
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.0013
.0012
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.0010
.0009
.0014
.0013
.0012
.0011
.0010
.0009
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency

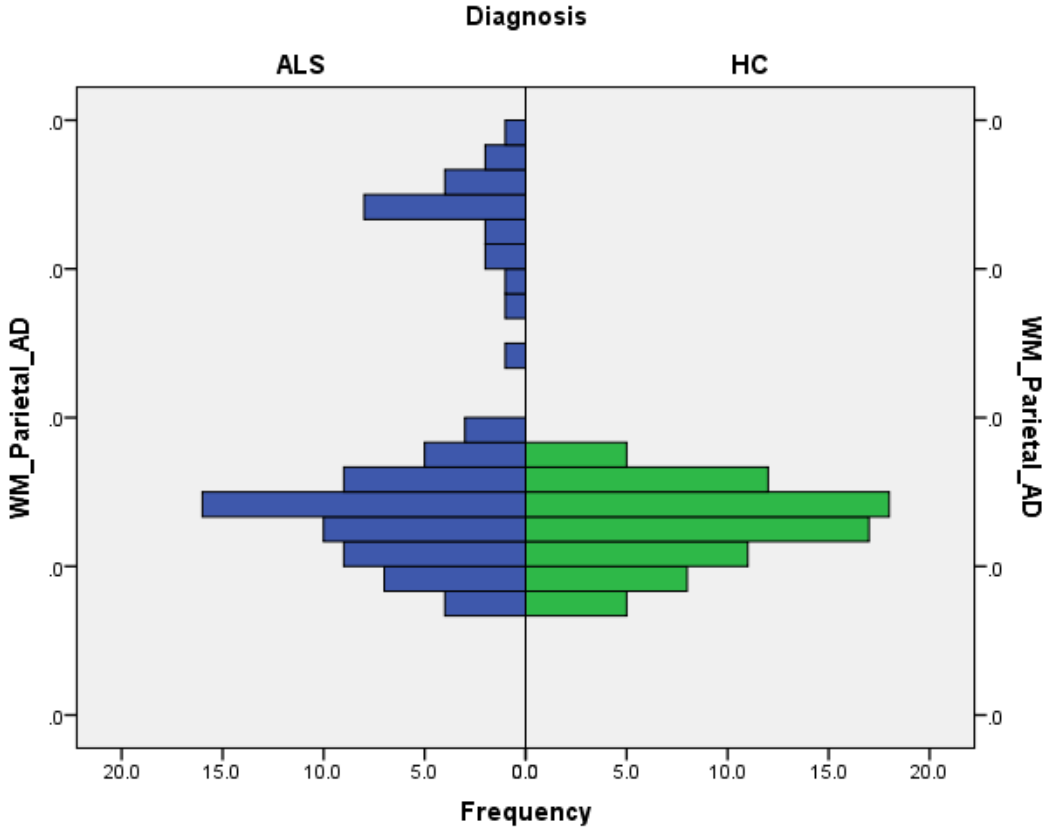
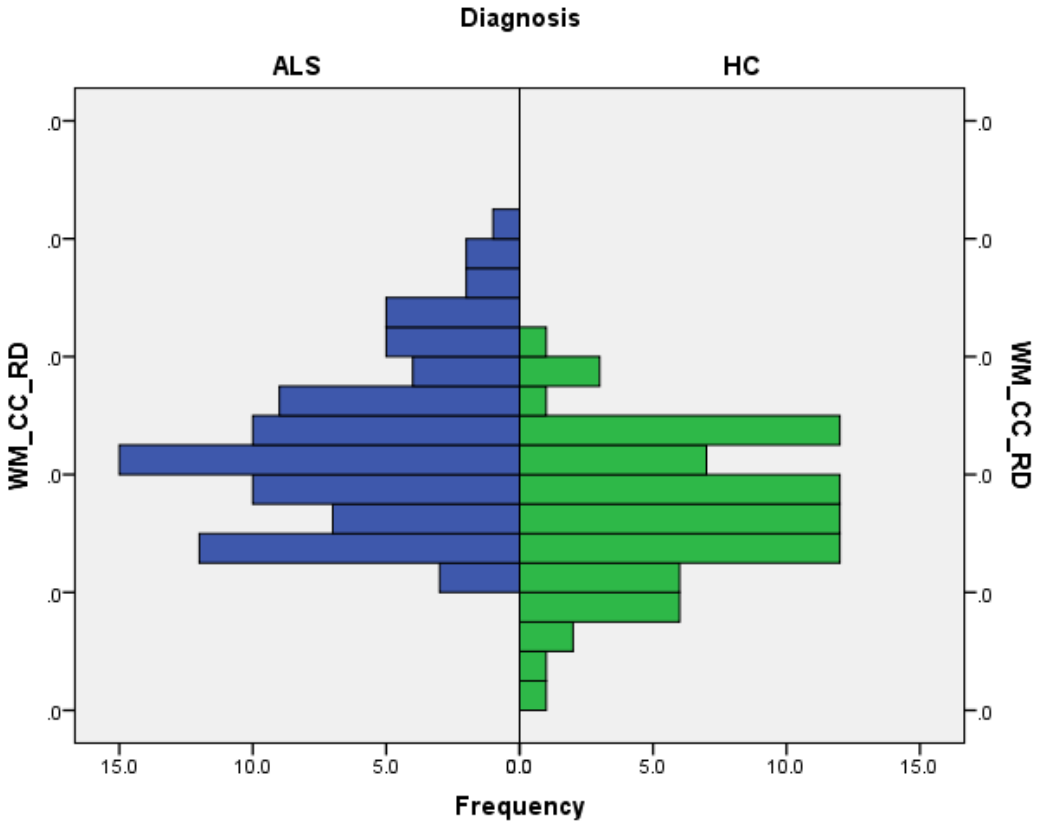
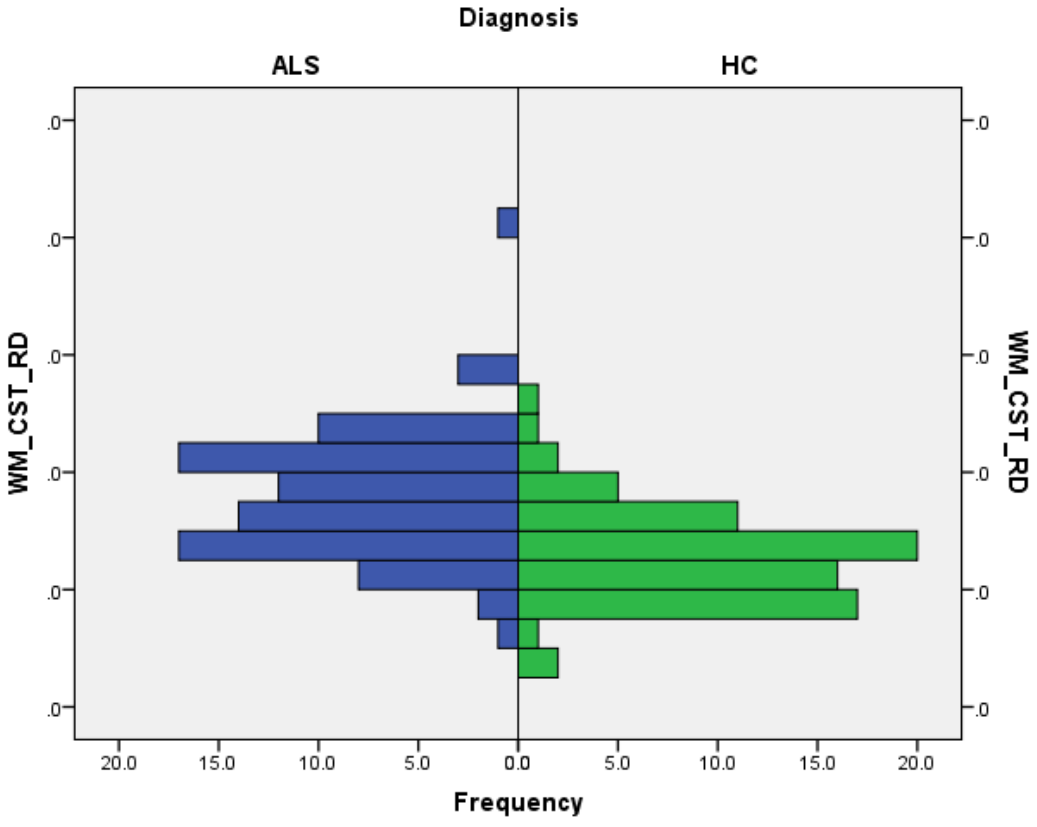


FIGURE 16: *Histograms of axial diffusivity (AD) in ALS patients and healthy controls*


Diagnosis
ALS
HC
WM_CC_RD
WM_CC_RD
.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
Frequency


Diagnosis
ALS
HC
WM_CST_RD
WM_CST_RD
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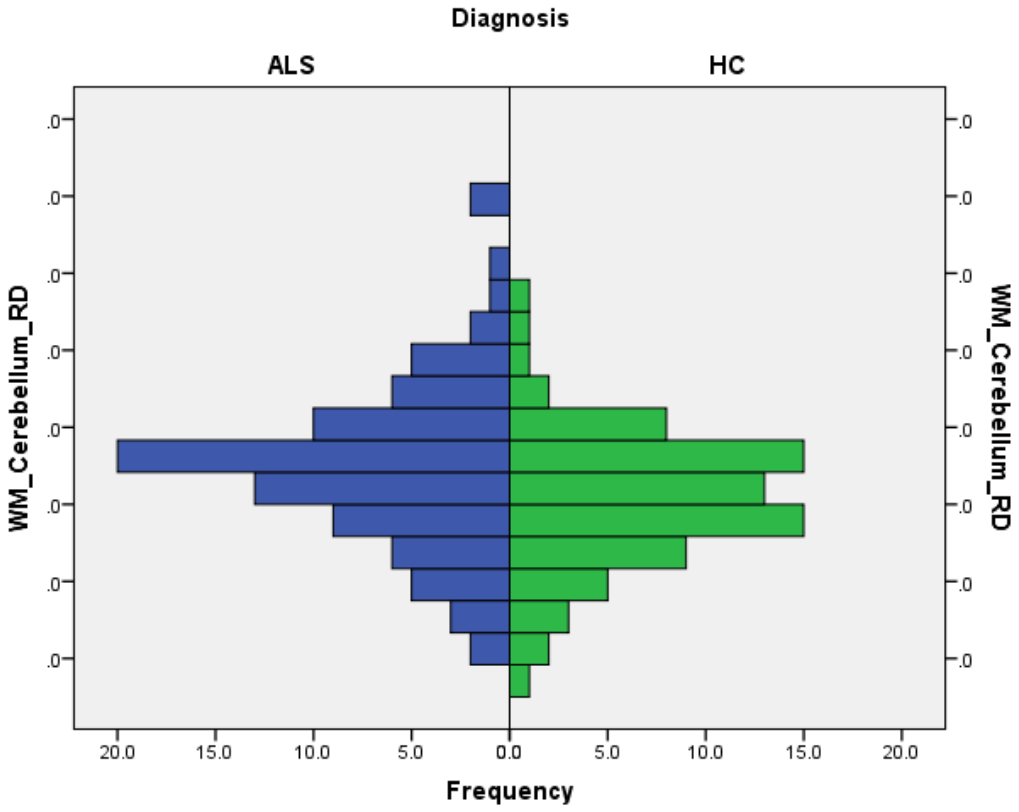
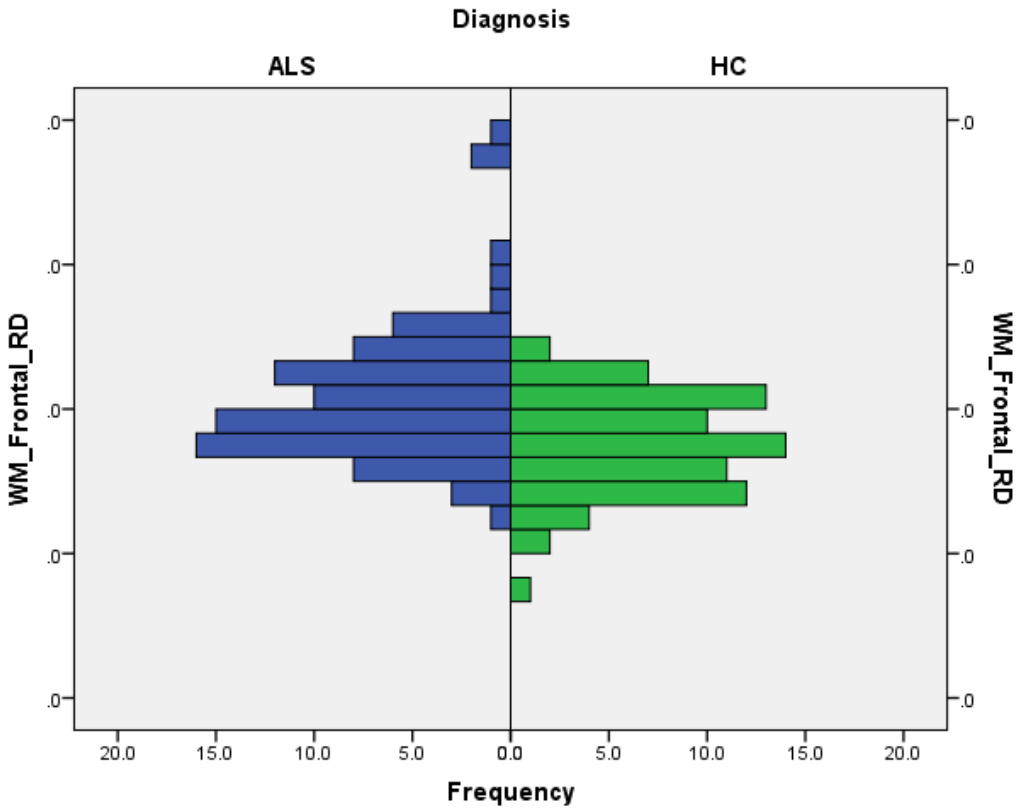
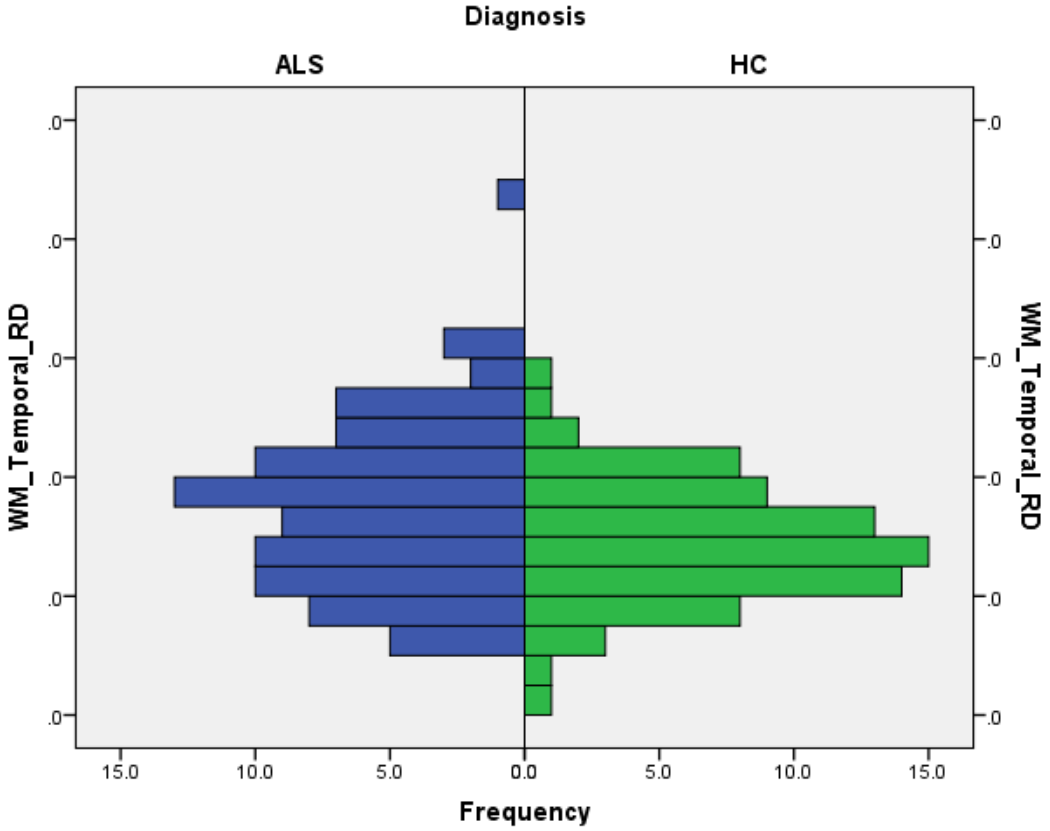
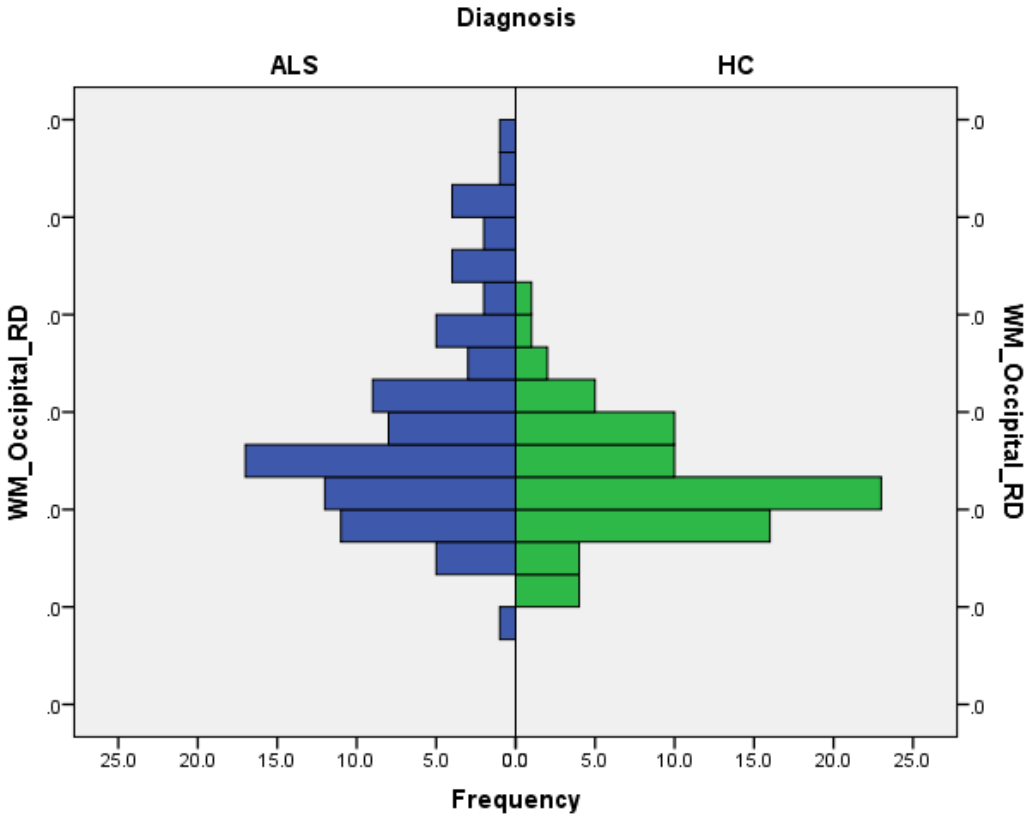
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Cerebellum_RD
WM_Cerebellum_RD
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Frontal_RD
WM_Frontal_RD
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
.0
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
Frequency


Diagnosis
ALS
HC
WM_Temporal_RD
WM_Temporal_RD
.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
Frequency


Diagnosis
ALS
HC
WM_Occipital_RD
WM_Occipital_RD
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25.0
20.0
15.0
10.0
5.0
0.0
5.0
10.0
15.0
20.0
25.0
Frequency

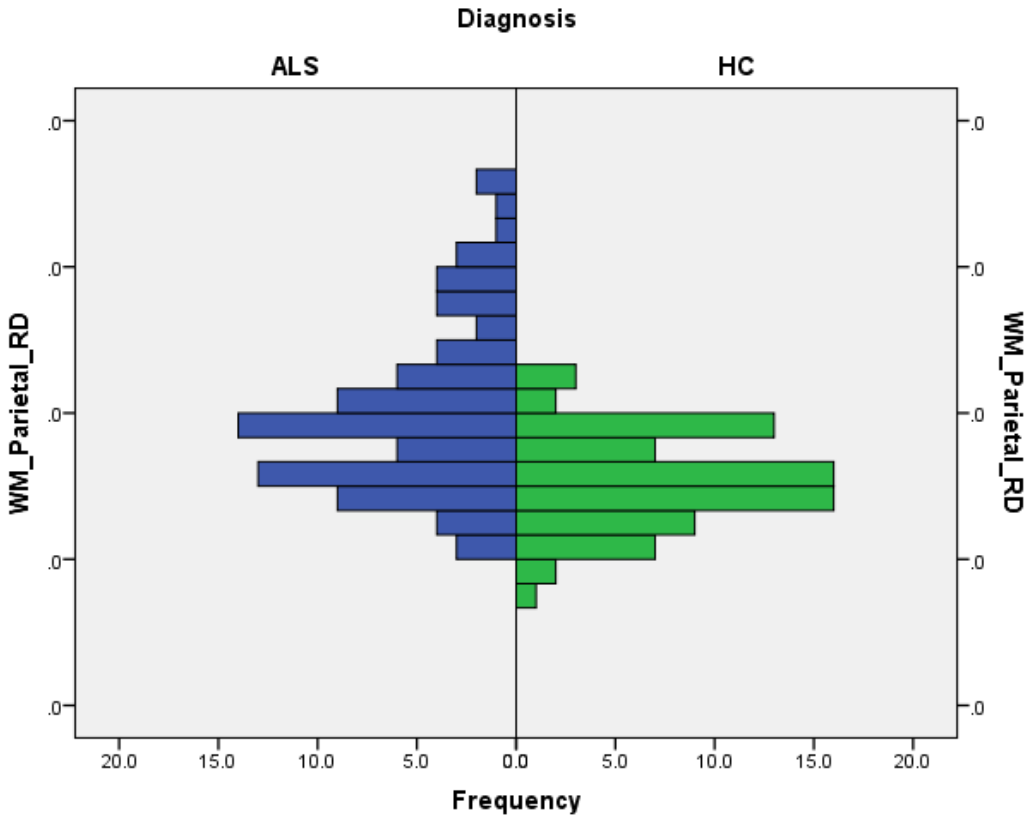

FIGURE 17: *Histograms of radial diffusivity (RD) in ALS patients and healthy controls*

CHAPTER 6: DISCUSSION

In this research study, multimodal imaging was used to characterise the imaging signature of ALS. While ALS has traditionally been associated with motor network degeneration this study was designed to also evaluate extra-motor involvement.

Multi-parametric diffusion tensor imaging is useful to characterise white matter pathology, as FA, AD, mean diffusivity (MD) and RD reflect on different aspects of white matter integrity and have differing detection sensitivity in ALS (332). MD and FA are composite measures of white matter integrity and are influenced by all three Eigen values (332). AD is generally regarded as a marker of axonal damage (333, 334), and RD is considered primarily a marker of myelin pathology (335, 336). ALS patients characteristically display reduced FA (56, 337, 338) which indicates alterations in white matter integrity, but FA does not distinguish the mechanism of neurodegeneration (339, 340). Increased MD(204), and increased RD have been associated with demyelination(336) but are not specific to inflammatory changes. Similarly, increased AD has been linked to axonal pathology (56, 204, 314) but few histopathologically validated imaging studies exist.

In this study we used both whole-brain and region of interest (ROI) analyses. The ROI approach involves the selection of specific brain regions a priori (341). This requires a clear hypotheses of the location of pathology (341). Voxel-based morphometry (VBM) is a robust method used to evaluate patterns of grey matter pathology. VBM permits whole brain analyses, allowing the detection of focal atrophy without a priori hypothesis about which structure to evaluate (342). It is a robust and extensively validated technique (342, 343). TBSS is a widely used whole-brain white matter technique which uses non-linear image transformation, combining the strengths of tractography-based and voxelwise analyses (344). TBSS is associated with outstanding sensitivity, interpretability and objectivity and widely used in diffusion imaging studies (344).

In order to capture the complete anatomical spectrum of degeneration in ALS, the evaluation of several measures of both grey and white matter integrity is necessary (326). Multimodal studies also facilitate the investigation of more novel imaging approaches through cross-validation against more established techniques (345).

Pathology in motor areas is well established in ALS and our grey matter analyses confirmed this in vivo. Considerable grey matter changes are seen in the right pre-central gyrus in our VBM analysis, which is in line with previous VBM studies in ALS demonstrating grey matter atrophy in the motor cortex (346-350), with several studies showing changes in the right pre-central gyrus (347, 348, 350). Extrapyramidal(351) and cerebellar(352) dysfunction also contribute to motor disability and such changes have consistently been detected on neuroimaging (197, 202). Grey matter atrophy was detected in our ALS patients in the cerebellum, but it did not reach statistical significance. However, cerebellar white matter is significantly affected in our cohort, as demonstrated by reduced FA and increased RD.

It is widely recognised that ALS is not just a disorder of the motor system, but a disorder of multiple systems (194). Our ROI analyses found a reduction in grey matter volumes in the frontal and temporal lobes. Atrophy in frontal and temporal lobes has been previously demonstrated (258, 261) and frontal lobe pathology has been linked to deficits in cognition (346, 353, 354). In our study, reduced FA, increased AD and increased RD were detected in both the frontal and temporal lobes. A bimodal distribution is seen in the histograms of grey matter volumes, FA and RD in the frontal lobe, but the same is not observed in the temporal lobe. There is a group of patients with significantly reduced grey matter volumes and FA in the frontal lobe and this may represent patients with early stages of cognitive impairment.

On VBM, asymmetrical changes are observed in the frontal and temporal lobes. There are changes in the right superior frontal gyrus, orbitofrontal area, and the orbital gyri.

Changes in the left inferior frontal gyrus which is the location of Broca's area are also observed. In the temporal lobe, there is evidence of changes in the grey matter of the right inferior temporal gyrus, the superior temporal gyri which are the location of Wernicke's area and the primary auditory cortex and the middle temporal gyri thought to be involved in language and semantic memory processing. These changes are consistent with studies which show impairments in language and visual recognition (355) and pathology in Broca's and Wernicke's area (356, 357). The parahippocampus and right hippocampus which have important roles in memory and learning as well as the caudate nuclei also show atrophic changes on VBM. Changes in subcortical grey matter structures in ALS-FTD have been observed in other studies (358) and these changes have been linked to specific neuropsychological deficits (359).

Autonomic (266), olfactory (267), sensory (265)and visual dysfunction are infrequently described in ALS but have been observed and documented by clinicians (194, 268). There is evidence of subtle sensory pathology on neuroimaging (360). We found reductions in grey matter volume in the postcentral gyrus in our ROI analyses. Some studies report pathological changes in the post-central gyrus (311) while others report preservation of the post-central gyrus (194). Our results show there is a subgroup of patients with much lower GM volumes in the post-central gyrus, indicating that there may be a subset of ALS patients with sensory involvement. Sensory impairment is not routinely evaluated in ALS despite patients reporting paraesthesias. Given the extensive literature of thalamic pathology in ALS and our finding of post-central gyrus involvement further studies focusing on sensory networks may be warranted.

While occipital and parietal involvement is inconsistently reported (194, 204), pathology in the parietal and occipital lobes has been previously reported on structural (257, 277), diffusion (278), functional MRI (263, 279) as well as susceptibility weighted imaging (SWI) (280) and MR spectroscopy (281). We have identified significant GM reductions in the occipital and parietal lobes. We also found reduced FA, and increased RD in the occipital and parietal lobes. AD was also significantly increased in the parietal

lobe. Our histograms reveal greater variability of all ROI metrics in ALS patients in comparison to healthy controls in the occipital and parietal lobes. There is a subgroup of patients with much lower GM volumes and FA and higher AD and RD in both the occipital and parietal lobes, indicating that subclinical or underreported visual and sensory impairments may exist in ALS. Further is needed to characterise these regions in further detail, and patients need to be assessed for visuospatial and sensory impairments.

On TBSS, changes in FA, RD and AD in the corpus callosum are detected. The corpus callosum is an established site of ALS-associated pathology. This is confirmed by the significant FA reduction identified by our ROI analyses and the significant increase in RD. Contrary to previous studies, we have not detected significant AD alterations. Reduced FA has been detected in previous studies in the body, genu and splenium of the corpus callosum in patients with ALS (361). The body of the corpus callosum has been previously shown to have increased AD (362) and RD (340, 363) as well as reduced FA (199, 340).

Several studies have previously reported increased RD (314, 340, 362) and reduced FA (199, 314, 337, 340, 364-366) in the corticospinal tracts (CST). On our TBSS analyses, FA, AD and RD of the CST are all affected in patients. Our ROI analyses showed that FA is significantly decreased and RD is significantly increased in the CST, but no significant differences were detected in AD between patients and controls. A bimodal distribution is seen in histograms for FA and RD in the CST. There is increased variability in FA in patients, with a significant number of patients with lower FA values in the CST.

TBSS also reveals superior longitudinal fasciculi pathology which are involved in regulating motor behaviour, perception of visual space, and processing somatosensory information and the inferior longitudinal fasciculi involved visual modality, including object, face and place processing, reading, lexical and semantic processing, emotion

processing, and visual memory, as well as forceps major and forceps minor are also affected. Changes in white matter metrics in the superior (39, 367) and inferior (39, 313) longitudinal fasciculi have been previously observed. Asymmetry is seen in some of the white matter metrics. There are changes in AD and RD in the right inferior longitudinal fasciculus and in AD the right uncinate fasciculus. Asymmetrical imaging findings are commonly found in ALS, but their significance is often not comprehensively discussed. (326) Few studies correlate unilateral imaging findings with the sidedness of symptoms on examination. Asymmetry is considered to be an established early-stage feature of ALS. (368) It is generally recognised that the disability profile, handedness, sample size and disease duration may be important factors in the asymmetry of imaging findings. (326)

Strengths

This is a prospective, case-controlled study. The study is a single-centre study eliminating the need for cross-platform harmonisation and standardisation of assessments between centres. Differences between ALS patients and controls were detected on observed independent quantitative structural and diffusion imaging metrics. Variables such as age and gender were accounted for. Both whole-brain and region of interest analyses were performed and detected significant differences between patients and controls. This study detected pathology in motor areas as expected, but it also highlights how extensive the extra-motor changes are in ALS.

Limitations

This study was conducted for this master thesis with a view of laying the foundations to prospective work. It was intended as a multi-parametric descriptive imaging study to characterise disease-specific signatures and allow myself as a M.Sc student to familiarise myself with the basics of study management, recruitment, patient

assessment and the basics of image analyses. It presents descriptive statistics only, and longitudinal analyses, receiver operating characteristics of ROIs, disease controls were not included. The study was limited to basic structural analyses and no cortical thickness, spectroscopy, or resting state fMRI data were analysed as part of this project. Given the main objective of the study, imaging patterns are described, but clinico-radiological correlations are not presented. The depths of the analyses and breadth of the study design was tailored to the M.Sc requirements and chosen to equip myself with basic clinical, statistical and neuroimaging skills.

Future directions

There is a large number of robust cross-sectional imaging studies in ALS, but to characterise anatomical patterns of propagation (196), detect dynamic structural and functional changes and evaluate compensatory processes (10, 14, 26, 28, 41, 193, 194), longitudinal studies are required. Longitudinal studies also have the potential to assess the predictive value of early-stage imaging features and validate putative prognostic markers.

Future studies can be improved by generating larger sample sizes in order to mitigate the effects of confounding factors. (326) In addition to healthy controls, inclusion of disease controls is essential in order to describe ALS-specific changes which differentiate ALS from other neurodegenerative conditions. Some imaging studies have begun to include disease controls (30-32). Imaging studies in ALS have already confirmed disease-specific pathological changes prior to symptom onset (183, 184). The pre-symptomatic disease-phase represents a crucial window for therapeutic and neuroprotective intervention. There is evidence of cognitive deficits, grey matter pathology and white matter changes (189) in presymptomatic *C9orf72* carriers and superoxide dismutase 1 (*SOD1)* mutation carriers also demonstrate metabolic changes

(182) before onset of motor symptoms. There is an urgent need for large prospective pre-symptomatic studies to further characterise this phase of the disease.

A number of clinical staging systems have now been developed, yet very few imaging studies report associations with staging (46) or neuropsychological performance (46, 47). Therefore there is a need for the standardised collection of clinical data in conjunction with MR data in order to perform correlation analyses between clinical findings and MR data.

Despite the widespread use of 3 Tesla systems, 7 Tesla platforms are increasingly available, and associated with improved contrast, resolution and signal-to-noise ratio. (369) Most imaging studies in ALS evaluate cerebral alterations (10). The technical challenges of spinal imaging such as physiological motion, small cross-sectional dimensions and susceptibility gradients have been gradually overcome (15-24) and there is a need for more studies which evaluate pathology in the spinal cord and the anterior horns specifically. Post-mortem pathological assessment of participants of ALS imaging studies could offer valuable insights regarding the sensitivity of ante-mortem imaging metrics and clarify what are the histopathological correlates of specific diffusivity indices. Post-mortem neuroimaging is another emerging trend which provides uniquely detailed structural images at high resolution thanks to dedicated pulse sequences with long acquisition times.

The majority of existing studies report ‘group-level’ observations, either disease-specific findings, genotype- or phenotype-associated changes. There is an increased need for the meaningful interpretation of individual scans in diagnostic, prognostic and monitoring applications. The need for individual data interpretation is increasingly addressed by emerging classification studies (192, 222).

CHAPTER 7: CONCLUSIONS

ALS is a highly heterogeneous condition due to variations in upper motor neuron and lower motor neuron involvement, extra-motor symptoms, age of onset, survival and progression rates. While clinical biomarkers are undeniably useful, neuroimaging measures are particularly promising biomarker candidates.

My hypothesis was that the multi-parametric analyses of cerebral structural and diffusion metrics will reveal focal cortical changes in the motor cortex, altered diffusivity in corticospinal tracts and corpus callosum and alterations in extra-motor regions in ALS in comparison to healthy controls. Cross-sectional clinical data were obtained and analysed for all newly recruited patients and the imaging data of 85 ALS patients and 76 controls were analysed. In this prospective case-controlled study, significant differences between ALS patients and controls were detected on observed independent quantitative structural and diffusion imaging metrics. Both our whole-brain and region of interest analyses detected pathology in motor areas as expected, but they also highlighted how extensive the extra-motor changes are in ALS, and such changes warrant further investigation.

The assessment of multiple imaging parameters is necessary in order to accurately and comprehensively characterise pathology in ALS. It is widely recognised that ALS is not just a disorder of the motor system, but a disorder of multiple systems. Our results show this, proving our hypothesis. The novelty of this work is the multifaceted characterisation of extra-motor pathology. While evidence of pathology in posterior regions of the brain in ALS has been inconsistently reported in previous studies, we have demonstrated extensive occipital and parietal pathology based on our ROI analyses. An additional novelty of our results is that we have captured post-central gyrus atrophy which is relatively under reported in ALS. Further studies focusing on sensory networks may be warranted. Furthermore, we highlight cerebellar white

matter degeneration which is difficult to detect clinically given the concomitant pyramidal tract degeneration.

Characterisation and recognition of extra-motor involvement in ALS has important implications for individualised patient care, caregiver support and the development of novel biomarkers. Multi-parametric neuroimaging has the potential to facilitate the classification of heterogeneous cohorts, and the monitoring of disease progression by tracking changes in selected ROIs overtime, thereby allowing the meaningful interpretation of individual scans in diagnostic, prognostic and monitoring applications.

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