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CEREBRAL CHANGES IN POLIOMYELITIS SURVIVORS: A COMPUTATIONAL NEUROIMAGING STUDY

A dissertation submitted to Trinity College Dublin for the degree of Doctor of Philosophy (PhD)

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Declaration

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Signed: 

Stacey Li Hi Shing
June 2022

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Glossary

101-PNR – 101-point numeric rating
10MWT – 10-meter walk test
2MWT – 2-minute walk test
6MWT – 6-minute walk test
AAA – Anterior amygdaloid area
ABN – Accessory basal nucleus
AD – Axial diffusivity
ALS – Amyotrophic lateral sclerosis
ALSFRS-r – Revised amyotrophic lateral sclerosis functional rating scale
AN – Attention network
ANCOVA – Analysis of covariance
ANOVA – Analysis of variance
ASO – Antisense oligonucleotide
AV – Antero-ventral
BDI – Beck depression inventory
BiPAP – Bilevel positive airway pressure
BMI – Body mass index
BN – Basal nucleus
bvFTD – Behavioural variant frontotemporal dementia
C9orf72 – Chromosome 9 open reading frame 72
CAS – cytokine analysis study
CAT – Cortico-amygdaloid transition
CBT – Cognitive behavioral therapy
CC – Corpus callosum
CeM – Central medial
CeN – Central nucleus
Chol – Choline
CK – Creatine kinase
CL – Central lateral
CM – Centromedian
CMAP – Compound muscle action potential
CMV – Controlled mechanical ventilation
CN – Cortical nucleus
CPC – Cortico-ponto-cerebellar tract
Cr – Creatine and phosphocreatine
CSA – Cross-sectional area
CSE – Clinical study extension
CSF – Cerebrospinal fluid
CSF-MC – cerebrospinal fluid mononuclear cells
CST – Corticospinal tract(s)
CTC – Cerebellothalamo-cortical tract
CTh – Cortical thickness
DKI – Diffusional kurtosis imaging
DMN – Default mode network
DRP – Dipeptide repeat protein
DTI – Diffusion Tensor Imaging
ECAS – Edinburgh cognitive and behavioural ALS screen
ELISA – Enzyme-linked immunosorbent assay
EMG – Electromyogram
EMM – Estimated marginal mean
ESS – Epworth sleepiness scale
FA – Fractional anisotropy
FBA – Fixed-based analysis
FDG – Fluorodeoxyglucose (18F)
FIS – Fatigue impact scale
FLAIR – Fluid-attenuated inversion recovery
FOV – Field of view
FrSBe – Frontal System Behavioural Scale
FSL – FMRIB Software Library
FSL-FAST – FMRIB's Automated Segmentation Tool
FSL-FLIRT – FMRIB's Linear Image Registration Tool
FSS – Fatigue Severity Scale
FTD – Frontotemporal dementia
FVC – forced vital capacity
FWE – Familywise error
GIF – Geodesic information flows
Glu – Glutamate
GM – Grey matter
GPEI – Global Polio Eradication Initiative
GRN – Progranulin gene

HADS – Hospital Anxiety and Depression Scale
HC – Healthy control
HDsEMG – High density surface electromyography
HHD – hand-held dynamometry
HSP – Hereditary spastic paraplegia
IASP – International Association for the Study of Pain
IBM-FRS – Inclusion body myositis functional rating scale
ICP – Inferior cerebellar peduncle
IPAP – inspiratory positive airway pressure
IR-SPGR – Inversion recovery prepared spoiled gradient recalled echo
IR-TSE – Inversion recovery turbo spin echo sequence
KAFO – Knee ankle foot orthosis
L-SG – Limitans/suprageniculate
LD – Latero-dorsal
LDDMM – Large Differmorphic Deformaton metric mapping
LEoP – Late effects of polio
LGN – Lateral geniculate
LIC – Lung insufflation capacity
LL – Lower limb
LMN – Lower motor neuron
LN – Lateral nucleus
LOSP – Late-onset sequelae of poliomyelitis
LP – Lateral posterior
Lt – Left
LVR – Lung volume recruitment
MAF – Multidimensional assessment of fatigue
MANCOVA – Multivariate analysis of covariance
MAPT – Microtubule-associated protein tau
MCP – Middle cerebellar peduncle
MD – Mean diffusivity
MD – Myotonic dystrophy
MDl – Mediodorsal lateral parvocellular
MDm – Mediodorsal medial magnocellular
MDT – Multidisciplinary team
MEP – Maximal expiratory pressure
MFI-20 – Multidimensional functional inventory
MFM scale – Motor function measurement scale
MGN – Medial geniculate
MIP – Maximal inspiratory pressure
MMPI – Minnesota multiphasic personality inventory
MN – Medial nucleus
MND – Motor neuron disease
MNI152 – Montreal Neurological Institute 152 standard space
MRC – Medical Research Council Scale for muscle strength
MRI – Magnetic resonance imaging
MRS – Magnetic resonance spectroscopy
MTA – mesial temporal lobe atrophy
MUAP – Motor unit action potential
MV – Minute ventilation
MV-re – Reuniens/medial ventral
MVA – Maximal voluntary activation
MVC – Maximal voluntary contraction
MVIC – Maximal isometric voluntary contraction
NAA – N-acetylaspartate
NCI – Neuronal cytoplasmic inclusions
NFL – Neurofilament light polypeptide/neurofilament light chain
NHP – Nottingham health profile
NII – Neuronal intranuclear inclusions
NIPPV – Nasal intermittent positive pressure ventilation
NIV – Non-invasive ventilation
NODDI – Neurite orientation dispersion and density imaging
OSA – Obstructive sleep apnea
PASE – Physical activity of the elderly
PBMC – peripheral blood mononuclear cells

Pc – Paracentral
pC9+ – Presymptomatic C9orf72-positive
PCF – unassisted peak cough flow
PCR – Polymerase chain reaction
PET – Positron emission tomography
Pf – Parafascicular
PFS – Piper fatigue scale
PFT – Pulmonary function test
PLMS – Periodic limb movements of sleep
PLS – Primary lateral sclerosis
PMC – Primary motor cortex
PN – Paralaminar nucleus
pNfH – Phosphorylated neurofilament heavy protein
PPL – Polio problem list
PPS – Post-polio syndrome
Pt – Paratenial
pTDP 43 – Phosphorylated TDP-43
PuA – Pulvinar anterior
PuI – Pulvinal inferior
PuL – Pulvinar lateral
PuM – Pulvinar medial
PV – Polio virus
qMRI – quantitative magnetic resonance imaging
QMT – Quantitative motor test
QSI – q-space imaging
QSM – Quantitative susceptibility mapping
rCT – randomized controlled trial
RD – Radial diffusivity
RDBPC – Randomized double-blind placebo controlled
REE – resting energy expenditure
RLS – Restless leg syndrome
RNA – Ribonucleic acid
ROI – Region of interest
RQ – respiratory quotient
RR – respiratory rate
Rs-fMRI – Resting-state functional MRI
Rt – Right
RT-PCR – Reverse transcription polymerase chain reaction
rTMS – Repetitive transcranial magnetic stimulation
S-SFEMG – Single fiber electromyography stimulation
SB – Southern blot
SBMA – Spinal-bulbar muscular atrophy
SCP – Superior cerebellar peduncle
SD – Standard deviation
SE-EPI – Spin-echo echo planar imaging
SENSE – Sensitivity Encoding
SF-36 – 36-item short form survey
SFEMG – Single fiber electromyography
SFQ – Short fatigue questionnaire
SIP – Sickness impact profile
SIPP – Self-reported impairments in persons with late effects of polio
SMA – Spinal muscular atrophy
SMN – Sensorimotor network
SMN gene – Survival motor neuron gene
SN – Salience network
SNIP – Sniff nasal inspiratory pressure
SOD1 – Superoxide dismutase 1
SPECT – Single-photon emission computer tomography
SPG4 – Hereditary spastic paraparesis type 4
SPIR – Spectral presaturation with inversion recovery
SPSS – IBM Statistical product and service solutions
SSRI – Selective serotonin reuptake inhibitors
SSS test – Sit-stand-sit test
SUVRs – standard uptake value ratios
SyN – Symmetric Diffeomorphic Image Normalisation method
T1w – T1-weighted imaging
TBI – Traumatic brain injury
TBM – Tensor based morphometry

TBSS – Tract based spatial statistics
tDCS – Transcranial direct current stimulation
TDP-43 – TAR DNA-binding protein 43
TE – Echo time
TFCE – Threshold-free cluster enhancement
TI – Inversion time
TIV – Total intracranial volume
TQNE – Turf 's quantitative neuromuscular examination
TR – Repetition time
TUG test – Timed-Up-and-Go test
UL – Upper limb
UMN – Upper motor neuron
UW-SES – University of Washington self-efficacy scale
VA – Ventral anterior
VAmc – Ventral anterior magnocellular
VAS – Visual analog scale
VAS-F – Visual analog scale for fatigue
VBM – Voxel-based morphometry
VCO_2 – carbon dioxide production
VLa – Ventral lateral anterior
VLp – Ventral lateral posterior
VM – Ventromedial
VO_2 – oxygen consumption
VPL – Ventral posterolateral
WBV – Whole body vibration
WHOQOL-BREF – World Health Organization quality of life abbreviated scale
WM – White matter
WPV – Wild poliovirus

Summary

Post-polio syndrome (PPS) is a slowly progressive LMN condition that affects 20-85% of polio survivors many decades after their initial acute infection. While motor dysfunction is the hallmark of the disease, extra-motor features such as generalised fatigue, decreased endurance, sleep disturbance, chronic pain, neuropsychological deficits, sensory impairments and cold intolerance have also been reported. PPS is a multi-system condition with considerable quality of life implications with no established aetiology, confirmed pathophysiology and patterns of cerebral involvement. There is a striking paucity of PPS post-mortem and imaging studies compared to other MND phenotypes and the evidence of cerebral involvement remains conflicting. PPS is a diagnosis of exclusion and the mainstay treatment remains supportive and no disease modifying therapy currently exists.

The main aim of this research study was the in-depth characterisation of PPS-associated cerebral changes using high resolution structural and diffusion MRI. Extra-motor cortical, subcortical and cerebellar involvement were specifically assessed. The associated brain changes were contrasted to cerebral involvement in amyotrophic lateral sclerosis (ALS), a mixed UMN/LMN motor neuron disease.

In this prospective, single-centre neuroimaging study, a total of 43 adult polio survivors were recruited, assessed using standardised clinical assessments and underwent high-resolution 3T MRI. The imaging profile of the adult polio survivors were compared to 117 healthy controls (HC) and 88 patients with amyotrophic lateral sclerosis (ALS). Whole-brain and region-of-interest morphometric analyses were undertaken to evaluate patterns of grey matter changes. Tract-based spatial statistics were performed to assess diffusivity alterations within the white matter skeleton. Furthermore, cortical thickness measurements, subcortical volumetry, brainstem, thalamic and amygdalar segmentation were performed. Cerebellar changes were evaluated using cortical thickness and volume analyses, morphometry and voxel-wise white matter analyses to appraise the cerebellar peduncles.

Adult polio survivors exhibited high levels of motor dysfunction affecting predominantly their lower limbs. Extra-motor features were detected in this cohort using our screening tools and included high prevalence of problematic fatigue, verbal fluency and language deficits and apathy. Poliomyelitis survivors exhibited increased grey matter partial volumes within the brainstem, occipital love and cerebellum accompanied by increased WM integrity in the corticospinal tract, cerebellum, bilateral mesial temporal lobes and inferior frontal tracts. Furthermore, polio survivors demonstrated increased cortical thickness within cerebellar lobules I,II and III of the right anterior lobe and lobules VIIIA and VIIIB bilaterally accompanied by enhanced cerebellar peduncular organisation particularly within the middle cerebellar peduncles. Limited atrophy were denoted within the cingulate gyrus, temporal pole and left nucleus accumbens when contrasted to healthy controls.

Overall, these findings suggest a structural cortical and cerebellar reorganisation in poliomyelitis survivors which may represent adaptive changes to severe lower motor neuron injury in early life and as a result of ensuing motor disability.

First Authored Peer-Reviewed Publications

Post-polio Syndrome: More Than Just a Lower Motor Neuron Disease
Li Hi Shing S, Chipika RH, Finegan E, Murray D, Hardiman O, Bede P.
Front Neurol. 2019 Jul 16;10:773.
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Increased cerebral integrity metrics in poliomyelitis survivors: putative adaptation to longstanding lower motor neuron degeneration
Li Hi Shing S, Lope J, McKenna MC, Chipika RH, Hardiman O, Bede P.
J Neurol Sci. 2021 May 15;424:117361.
DOI: 10.1016/j.jns.2021.117361.

Imaging data indicate cerebral reorganisation in poliomyelitis survivors: Possible compensation for longstanding lower motor neuron pathology
Li Hi Shing S, Lope J, Chipika RH, Hardiman O, Bede P.
Data Brief. 2021 Aug 21;38:107316.
DOI: 10.1016/j.dib.2021.107316.

The imaging signature of C9orf72 hexanucleotide repeat expansions: implications for clinical trials and therapy development
Li Hi Shing S, McKenna MC, Siah WF, Chipika RH, Hardiman O, Bede P.
Brain Imaging Behav. 2021 Oct;15(5):2693-2719.
DOI: 10.1007/s11682-020-00429-w.

Extra-motor manifestations in post-polio syndrome (PPS): fatigue, cognitive symptoms and radiological features
Li Hi Shing S, Lope J, Chipika RH, Hardiman O, Bede P.
Neurol Sci. 2021 Nov;42(11):4569-4581.
DOI: 10.1007/s10072-021-05130-4.

The neuroradiology of upper motor neuron degeneration: PLS, HSP, ALS
Li Hi Shing S, Bede P.
Amyotroph Lateral Scler Frontotemporal Degener. 2022 Feb;23(1-2):1-3.
DOI: 10.1080/21678421.2021.1951293.

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Li Hi Shing S, Murad A, Lope J, Hardiman O, Bede P.
J Integr Neurosci. 2022 Mar 23;21(2):65.
DOI: 10.31083/j.jin2102065.

The Diagnosis and Management of Motor Neurone Disease (book chapter)
Li Hi Shing S, Lope J, Bede P.
Living with Motor Neurone Disease - A complete guide 2021 (pp. 31–46). essay, Cork University Press.

1 Post-polio syndrome: A systematic review of the existing literature

1.1 Introduction

Poliomyelitis was one of the most acutely debilitating infections of the 20th century that affected millions in the 1940s and 1950s and more recently in India during an outbreak in 1988 [1]. Following the introduction of the polio vaccine in the mid-1950s and early 1960s, there has been a dramatic decline in the number of new polio cases and it is estimated to be 99% eradicated today. Despite the enormous progress in the eradication of the polio virus, 15-20 million people across the world still suffer from the sequelae of the infection [2]. A large proportion of polio survivors has been presenting with a constellation of new neurological symptoms that has been described as Post-Polio Syndrome (PPS). The description of PPS is attributed to Jean-Martin Charcot in 1875 but was only widely recognised by the medical community in the early 1980s [3]. PPS is characterised by new neurological deficits after a long period of neurological stability, typically at least 15 years after the initial polio infection. PPS may manifest as new, persistent, and progressive muscle weakness, atrophy, limb fatigability, myalgia, arthralgia and dysphagia, but also as generalised fatigue, which typically has a considerable impact on the patients' quality of life. The estimates of the percentage of polio patients affected by PPS are inconsistent, varying between 20 – 85% [4, 5] depending on the diagnostic criteria applied [2]. As a result, despite the rarity of acute polio infection in the modern world, PPS is likely to persist for the next few decades. Despite its prevalence, post-polio syndrome remains surprisingly under-researched and poorly characterised. The purpose of this review is to provide a comprehensive overview of the aetiological, genetic, diagnostic, prognostic factors and treatment modalities in PPS while highlighting key gaps that require further research.

1.2 Methods

A literature search was performed on PubMed using the search term "post-polio syndrome", "postpolio syndrome" or "post polio syndrome" alone and in combination with "epidemiology", "pathophysiology", "clinical features", "fatigue",

"neurophysiology", "brain imaging", "electromyography", "inflammation", "diagnosis", "management", "clinical trial", "longitudinal", "cross-sectional", "case report", "autopsy" and "post mortem". Only articles written in English and published between January 1980 and May 2019 were selected for literature review. Identified publications were categorised into "academic" papers discussing pathophysiology, genetic susceptibility, biology, and "clinical" papers focusing on diagnostic criteria, management, rehabilitation and clinical trials.

1.3 Results

1.3.1 Pathophysiology

During the acute poliomyelitis infection, 95% of those infected remain asymptomatic or only suffer flu-like symptoms while the remaining 5% succumb to the paralytic form of the disease. Acute poliomyelitis is typically spinal, affecting the limbs and respiratory musculature, but bulbar manifestations affecting speech and swallow are also well-documented. Polioenterovirus type 1 is the main cause of meningeal, spinal cord and brain inflammation as it can cross the blood-brain barrier independently from poliovirus receptors [6, 7]. Ensuing anterior horn degeneration, and apoptosis post infection has been widely recognised as the hallmark feature of paralytic poliomyelitis. Following the acute phase, axonal sprouting takes place reinnervating the muscle of the affected regions [8, 9]. Motor units gradually become abnormally enlarged, up to seven-fold their original size [10] rendering them metabolically unsustainable [11]. This process can take up to three decades from the acute infection to the development of PPS symptoms [12]. The concomitant denervation-reinnervation process is evidenced by electromyography (EMG) findings [13-17] and muscle histology showing small angulated fibres [18, 19] and muscle fibre type-grouping [15]. Metabolic stress [11, 20], overuse [21, 22], physiological ageing [20, 23] and persistent inflammation [24] are also thought to contribute to gradual motor unit failure. Motor units loss has been consistently correlated to functional decline in longitudinal studies [13, 14, 25, 26]. Overuse of functioning muscle units is thought to induce detrimental structural alterations [27, 28]. Cellular adaptation in the muscles, such as fibre alteration from type II (fast) to type I (slow) [28], changes in contractile properties [29-31], and muscle hypertrophy [9] are likely to contribute to muscular fatigue and myalgia in PPS. The persistence or reactivation of polio virus in polio survivors has also been suggested with conflicting reports. Two

research studies [7, 32] have identified polio-virus (PV) genomic sequences in the CSF and peripheral leucocytes as well as high serum IgM anti-PV antibody titres, which were absent in stable polio survivors and in other neurodegenerative groups [33]. Other studies however could not confirm these findings [34]. An inflammatory or autoimmune basis to post-polio syndrome has also been proposed. This hypothesis originates from post mortem observations of inflammatory changes in the spinal cord of PPS patients [35, 36]. The role of inflammation is also supported by in vivo evidence. Increased serum and CSF levels of pro-inflammatory cytokines and peptides such as TNF-α, IFN-γ were repeatedly observed in PPS [37-39]. Furthermore, TNF-α and IFN-γ levels respond to IVIg therapy in PPS, and remain unchanged in controls [37, 38, 40]. However, no correlations have been detected between symptom severity [38], rate of decline [37] and pro-inflammatory peptide levels. Skeletal muscle biopsies also exhibit inflammatory changes and increased expression of prostaglandin E2 synthetic pathway enzymes [41]. Relatively limited evidence exists to support the autoimmune basis of PPS. One study identified high titres of PV antibodies concurrently with high levels of regulatory T cells [42], while another study [43] found normal levels of immune complexes in PPS patients. No specific anti-muscle or anti-neuronal autoantibodies have been associated with PPS [44]. A genetic predisposition for PPS has also been investigated, but no conclusive risk profile has been identified to date. *SMN* gene deletion [45, 46] associated with spinal muscular atrophy (SMA) was not reported in PPS, but Fc-gamma receptor IIIA polymorphisms may play a role in the predisposition to PPS [47].

1.3.2 Neuropathology and neuroimaging

Post-mortem studies are conflicting with regards to cerebral involvement in post-polio syndrome. Post-mortem studies [48] from 50-70 years ago suggest that polio virus preferentially affects the reticular formation, posterior hypothalamus, thalamus, putamen, caudate, locus coeruleus and substantia nigra which may account for the late-onset fatigue and attention deficit [49-52]. Interestingly, cortical involvement is relatively selective and preferentially involves the precentral gyrus and pre-motor areas. A more recent case report [53] and a retrospective analysis of formalin-fixed central nervous system (CNS) tissue of a small cohort of patients [33] arrived at a different conclusion. They identified no cerebral involvement at all, but selective spinal cord pathology affecting the anterior roots with dorsal root sparing. These studies detected enterovirus

RNA in spinal cord only. There have also been rare reports of polio patients developing ALS with characteristic histopathological findings [54, 55]. Compared to other motor neuron diseases [56], there is a striking paucity of brain [57] and spinal cord imaging studies in PPS [58]. Magnetic resonance imaging (MRI) has been used to evaluate volumetric changes [59] and to correlate anatomical changes to post mortem findings [48]. The main focus of existing brain imaging studies in PPS was to explore the substrate of fatigue. Multiple hyperintensities were identified in the reticular formation, putamen and medial lemniscus in the majority of PPS patients [48] which is consistent with previous post mortem studies [49-52]. A large study of 118 participants compared the brain volume profile of 42 PPS patients, 49 multiple sclerosis patients and 27 controls, and no statistically significant volume reductions were identified in PPS [59]. No association was identified between fatigue and brain volumes. The majority of existing studies are cross-sectional which provide limited insights into progressive longitudinal alterations [60]. There is an ongoing longitudinal, case-control study to characterise spinal cord alterations in PPS [61].

1.3.3 Diagnosis

Post-polio syndrome is a clinical diagnosis, supported by electrophysiological findings and possible mimics need to be reassuringly ruled out. An extensive work-up including laboratory tests, imaging studies, cerebrospinal fluid sampling, detailed electrophysiological evaluation and muscle biopsies may be required to exclude alternative diagnoses. The diagnostic criteria for PPS was first proposed by Halstead et al. in 1991 [62] and evolved over time to the current March of Dimes diagnostic criteria [63, 64] which include:

1. Prior paralytic poliomyelitis with evidence of motor neuron loss, as confirmed by history of the acute paralytic illness, signs of residual weakness and muscle atrophy on examination, or signs of denervation on EMG.
2. A period of partial or complete functional recovery after acute paralytic poliomyelitis, followed by an interval (usually 15 years or more) of stable neuromuscular function.
3. Gradual onset (rarely abrupt) progressive and persistent new muscle weakness or abnormal muscle fatigability (decreased endurance), with or without generalized fatigue, muscle atrophy, or muscle and joint pain. Onset may at times follow

trauma, surgery, or a period of inactivity. Less commonly, bulbar dysfunction or respiratory weakness occurs.

4. Symptoms that persist for at least a year.
5. Exclusion of alternative neuromuscular, medical, and orthopaedic problems as causes of symptoms.

PCR amplification of poliovirus RNA in the CSF is indicative of prior history of poliomyelitis [6, 7, 32] and the presence of pro-inflammatory cytokines may also be detected [39, 65]. Proteomic CSF markers such as gelsolin, hemopexin, peptidylglycine alpha-amidating monooxygenase, glutathione synthetase and kallikrein 6 have been proposed as diagnostic markers but supporting evidence from larger studies is lacking. On muscle biopsy, hypertrophic muscle fibres type I [66, 67], indicative of compensatory reinnervation and small angulated fibres, indicative of active denervation [19] may be observed. CSF sampling and muscle biopsy also allows the exclusion of other neuromuscular mimics. People with PPS typically undergo detailed spinal imaging to rule out alternative structural, neoplastic, compressive or inflammatory spinal aetiologies which could manifest in lower motor neuron dysfunction [58, 68-70]. Electromyography (EMG) is an invaluable tool to assess suspected post-polio cases, as it allows the confirmation of a prior history of poliomyelitis while excluding differential diagnoses [71]. A variety of EMG techniques have been used in post-polio research studies including single fibre EMG (SFEMG), high density surface EMG (HDsEMG) [72] and macro-EMG. Ongoing denervation can be detected on conventional EMG by the presence of fibrillation and fasciculation potentials and increased jitter on SFEMG in newly weakened muscles [73]. Needle EMG can also readily detect sub-clinically affected muscles in PPS [74]. EMG measures correlate well with muscle strength and endurance [75, 76]. While EMG provides important insights, EMG measures don't differ significantly between those with PPS and stable polio [77] and thus EMG is not regarded as an electrodiagnostic tool to confirm PPS [73]. PPS is therefore a clinical diagnosis supported by laboratory tests.

1.3.4 The spectrum of clinical manifestations

Post-polio patients characteristically experience new onset muscle weakness, decreased endurance, muscle atrophy, myalgia, and fasciculations [78]. Additional symptoms often

include generalised fatigue, cold intolerance, dysarthria, dysphagia, and respiratory compromise [79, 80]. New symptoms typically occur in previously affected areas but sub-clinically affected body regions can also get affected [74]. Ambulatory difficulties often necessitate assistive devices and may lead to increased fall risk [81]. PPS is also associated with a wide range of non-motor symptoms. Frank sensory deficits may be detected, and paraesthesia are often reported by PPS patients. Changes in sensory evoked potentials have been linked to cord atrophy on MRI [82]. There have been consistent reports of cognitive deficits [83] in PPS including word finding difficulties [84], poor concentration, limited attention, memory impairment [85] and mood disturbances [86]. The non-motor aspects of PPS are often under evaluated despite their considerable quality of life implications [87]. Due to the combination of motor disability [88] and non-motor symptoms, many patients engage less in social activities [89] which may lead to social isolation. Generalised fatigue is one of the most distressing sequelae of PPS which is likely to be multifactorial due to muscle unit pathology, weight-gain, respiratory compromise, polypharmacy and poor sleep. **Figure 1-1**. The identification of the key 'fatigue-factors' in individual patients is indispensable for the effective pharmacological and non-pharmacological management of fatigue. Fatigue is thought to exhibit circadian variations and progress throughout the day [90]. Sleep disorders such as restless leg syndrome (RLS) [87, 91-94], sleep related breathing disturbances [95], obstructive sleep apnoea (OSA) [96], excessive daytime somnolence (EDS) and periodic limb movement in sleep (PLMS) [97] are not only often reported in PPS but they are likely to play an important role in the pathogenesis of fatigue in PPS [98, 99]. Fatigue is thought to be more severe in PPS with RLS, and correlate to the severity of RLS [87]. The simultaneous onset of RLS and PPS symptoms [91] and the positive response to pramipexole in an uncontrolled trial by Kumru et al. [93] have been interpreted as a pathophysiological link between RLS and PPS. [98] The putative link between RLS and neuroimmunological alterations [100, 101] may also suggest shared pathophysiological processes between PPS and RLS [99]. Furthermore, a higher incidence of cauda equina syndrome [102] and renal impairment [103] has also been reported in PPS but the association between these syndromes remains to be elucidated.

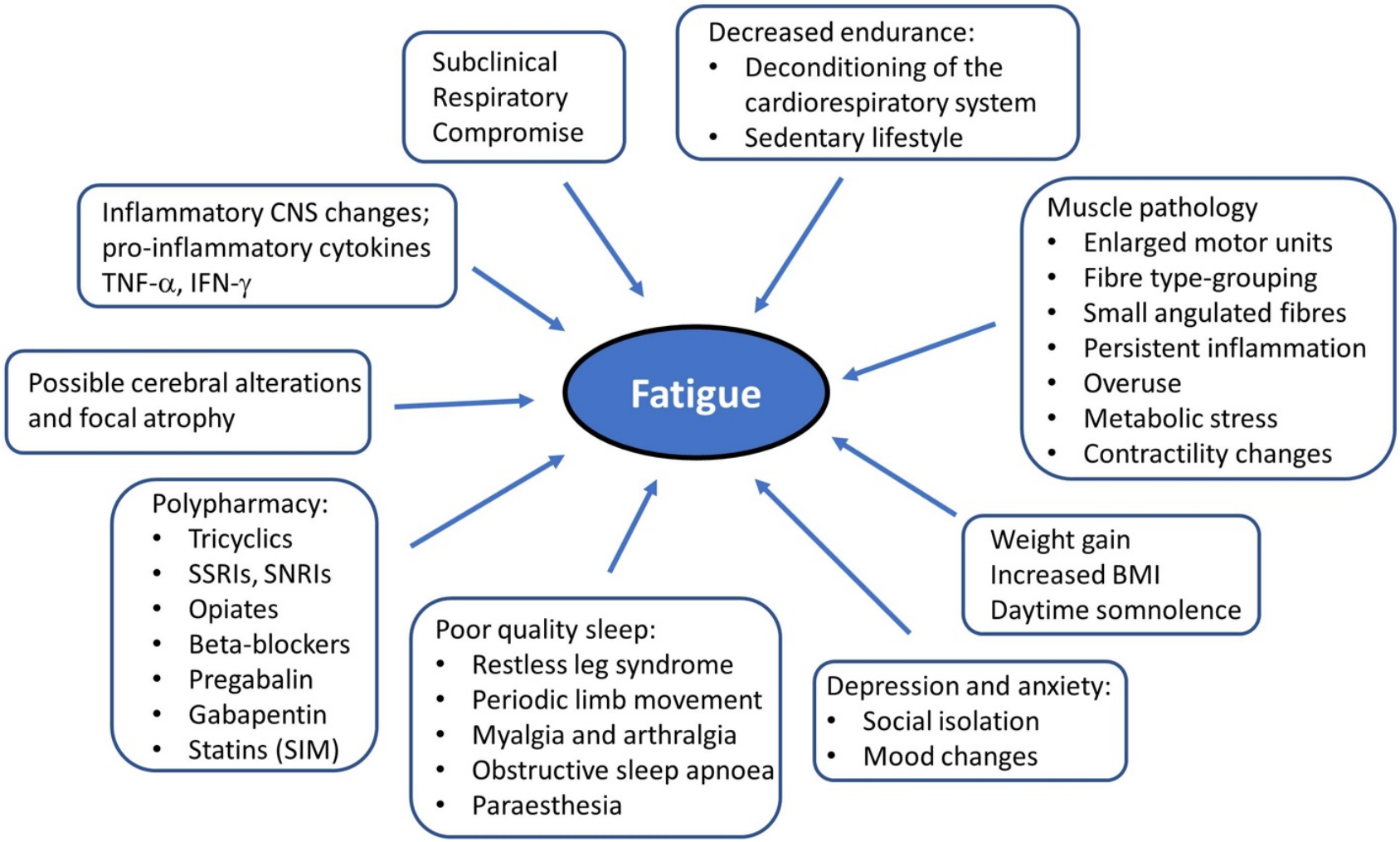

Figure 1- 1 Putative factors in the aetiology of generalised fatigue in post-polio syndrome.

1.3.5 Progression, Assessment and Monitoring

The majority of longitudinal studies [14, 25, 104-107] detect progressive muscle weakness, which contributes to deteriorating gait performance [107] and declining mobility [105]. Quantifying the rate of decline in PPS is challenging and no reliable functional predictors have been validated. Male gender is thought to be a negative prognostic indicator [108], but PPS is more common in females [12]. Most PPS patients who participated in research studies have lived with PPS for over 13 years suggesting that PPS is a relatively slowly progressive condition. There have also been however sporadic reports of rapidly progressive and life-threatening forms of PPS [109], which raises the question of occasional misdiagnoses or a link between PPS and amyotrophic lateral sclerosis (ALS) [54]. The severity of PPS-associated disability is typically evaluated clinically but a number of rating scales and questionnaires have been developed and validated for both clinical and research use. In addition to mobility and dexterity, these instruments evaluate the non-motor aspects of the condition such as fatigue, pain, sleeping disturbances and mood [110]. Clinical tests used to assess motor disability include the 6-minute walking test (6MWT) [111] at self-preferred speed, the 2-minute walking test (2MWT) at maximal speed [112], Timed-Up-and-Go test (TUG) [113], 10 metres walking test (10MWT), Sit-Stand-Sit test (SSS) [114]. Muscle strength is typically appraised by manual muscle testing using the MRC scale, or more objectively using a dynamometer during maximal isokinetic and isometric voluntary contraction. Endurance is measured using isometric contraction peak torque, isometric endurance, tension time index (TTI) or recovery of torque after endurance test [76]. Quantitative muscle mass assessment can be performed using ultrasound parameters such as muscle echo intensity and muscle thickness which are non-invasive tools for disease monitoring [115]. The most commonly used instruments to assess non-motor domains include the Fatigue Severity Scale (FSS) [116], Fatigue Impact Scale (FIS), Piper Fatigue Scale (PFS), Short Fatigue Questionnaire (SFQ), Nottingham Health Profile (NHP), Physical activity scale for the elderly (PASE) [117], Polio Problem List (PPL), Visual analogue scale (VAS) [118], Multidimensional Fatigue Inventory (MFI-20) [119], World Health Organisation quality of life abbreviated scale (WHOQOL-BREF) [120], University of Washington Self-Efficacy Scale (UW-SES) [121], Sickness Impact Profile (SIP), 36-item Short Form Health Survey (SF-36) [112]. Sleep disturbances [97] and respiratory function can be formally assessed through polysomnography and pulmonary function tests (PFT) [122,

123]. RLS is typically diagnosed clinically [124] and most commonly evaluated using the validated international RLS rating scale (IRLS) [87, 93, 125]. Maximal inspiratory and expiratory pressures (MIP and MEP), sniff nasal inspiratory pressure (SNIP) [126] and arterial blood gases are validate markers of respiratory function in PPS.

1.3.6 Non-pharmacological interventions

The effective management of the heterogeneous symptoms of PPS requires individualised care in a multidisciplinary setting [127]. Expert input from physiotherapists, occupational therapists, speech and language therapists, respiratory physicians, podiatrists, psychologists, dieticians, pain specialists, social workers, nurse specialists and orthotists are needed to meet the multifaceted care and support needs of PPS patients [128]. Individualised lifestyle modifications and energy conservation strategies are indispensable in the effective management of PPS [129]. PPS-specific training regimens alternating active intervals and rest have been developed to improve cardiorespiratory fitness, conserve energy during routine activities and maintain independence [130]. Isokinetic, isometric, resistance and endurance training are thought to improve muscle strength and endurance without further muscle unit degeneration [131-140]. Combining aerobic and flexibility training is also thought to improve QoL. Supervised training is advised in those with significant disability [141]. Training in a warm environment may have longer lasting effects than training in colder temperatures [142]. Patients with arthralgia may benefit from dynamic water exercises [143] as well as exercising in a group setting [144]. Deconditioning of the cardiorespiratory system [145] may limit the effectiveness of aerobic training in PPS [146], therefore aerobic regimens must be carefully tailored to individual fitness levels [147]. While some studies show improved endurance following mid- to high-intensity aerobic exercises [139, 140], a recent study [148] highlights that high-intensity aerobic exercise may not be beneficial in PPS patients with fatigue. Due to the heterogeneity of disability profiles in PPS, individualised training regimes and exercises that don't rely on anti-gravity strength are particularly important [148-150]. Home-based arm ergometry for example is a well-tolerated and safe form of aerobic exercise [149, 150]. Whole body vibration (WBV) has been proposed as an alternative to exercise in PPS [151] and improved mobility was reported in a small study [152], but no improvement was noted in muscle strength or gait performance [153]. Orthoses are commonly prescribed for PPS patients to improve mobility and reduce pain.

New powered-type Knee Ankle Foot Orthosis (KAFOs) offer limited benefits on gait symmetry or walking speeds but were shown to improve base support, swing time, stance-phase and knee flexion during swing phase [154]. The emergence of novel, light-weight materials such as carbon fibre [155] and the biomechanical analysis of individual walking patterns have helped to optimize orthosis-design for patients. The use of MIG3 Bioceramics fabrics for example had beneficial effects on pain and periodic limb movement [156]. Other lifestyle modification such as weight loss, smoking cessation, increased physical activity and modification to daily activities have all been beneficial to patients with PPS [22]. There are sporadic reports that anodal transcranial direct current stimulation (tDCS) of premotor regions [157], repetitive transcranial magnetic stimulation (rTMS) of the left prefrontal cortex [158] and static magnetic fields [159] may ameliorate fatigue, improve sleep, reduce pain and even improve motor functions in PPS, but these studies have not been replicated. PPS patients with bulbar involvement require expert phonatory and swallowing assessments by a speech-and-language therapist [160] and careful follow-up. Instrumental modalities such as ultrasonography and videofluoroscopy [161] and clinical instruments [162] can be used to detect progressive bulbar dysfunction and appraise the risk aspiration. Compensatory swallowing techniques, dietician input for food consistency alterations, individualised speech therapy and laryngeal muscle training may be helpful in PPS patients with bulbar involvement [163]. PPS patients who suffer from respiratory compromise and sleep related breathing disorders benefit from lung volume recruitment (LVR) [164] and non-invasive ventilation (NIV) such as Bi-PAP [165] or nasal intermittent positive-pressure ventilators (NIPPV) [166]. Invasive ventilatory support with a tracheostomy is seldom required in PPS [167].

Addressing the non-physical aspects of PPS; mitigating psychological responses, emotional reactions, frustration and fear of falling are equally important aspects of multidisciplinary care [168]. Despite its positive effects on self-esteem [169], cognitive behavioural therapy (CBT) is not superior to standard multidisciplinary care in the treatment of fatigue [170-172]. Psychotherapy is primarily aimed at reducing anxiety, improving depressive symptoms [173], alleviating pain [174, 175] and enhancing subjective well-being [176]. Hope-oriented psychotherapy and encouraging participation in work [177] promote resilience in polio survivors and is associated with improved social functioning [178], satisfaction with social roles, improved quality of life and superior mental health [179]. Peer-support groups are also instrumental in buffering the

impact of a functional impairment on psychosocial well-being [180]. Furthermore, a reduction of physical demands at work and ergonomic adaptations at the workplace not only help PPS patients to maintain their occupational activities but enjoy their work [181]. Rehabilitation nurses also play an important role in the setting of realistic health goals, encouraging resiliency and providing emotional support [182].

1.3.7 Pharmacological trials

Several randomised controlled clinical trials (RCT) were conducted in PPS. **Table 1-1.** High-dose prednisone [183], amantadine [184] and modafinil [185, 186] showed no superiority to placebo in the management of fatigue. Prednisone therapy, showed a short-lived improvement in muscular strength but no meaningful functional improvement [183]. The evidence for the benefit of pyridostigmine therapy remains conflicting. Some studies [187] identified no benefit on muscle function while others reported a slight improvement in walking performance [188]. Co-enzyme Q10 supplements are thought to have no effect on muscle strength, endurance or fatigue in PPS [189, 190]. A small RCT of lamotrigine, demonstrated improvements in VAS, NHP and FSS suggesting that it may be beneficial to treat pain and fatigue and improve quality of life [191]. Given the inflammatory and autoimmune hypothesis of PPS pathogenesis, intravenous immunoglobulin has been extensively investigated for its potential therapeutic effects. Its benefit with regards to pain, muscle strength, physical functioning and quality of life is inconsistent. Improved pain control and overall vitality [192, 193] seem to be the main benefit of intravenous immunoglobulin (IVIg) treatment. Two small uncontrolled trials [38, 194] and two larger RCTs [40, 65] arrived to similar conclusions with regards to pain control and improvement in serum and CSF inflammatory markers. The main indicators for response to IVIg include severe pain, fatigue, less than 65 years of age, and paresis mainly affecting the lower extremities [194-196]. Studies are somewhat conflicting on its effect on muscle strength [65, 197]. These findings however encourage further large RCTs to establish the target PPS cohort for IVIg treatment, treatment intervals and dose optimisation. A single-centre, double-blind RCT trial of L-citrulline [198] is currently underway to investigate its effect on muscle metabolism and function. It is at clinical phase IIa and has proven to be of beneficial in muscular dystrophies in improving endurance in both aerobic and anaerobic exercise. The symptomatic management of non-motor symptoms in PPS also has considerable quality of life benefits. Restless leg

syndrome in PPS often responds to dopamine agonists such as pramipexole [93, 199]. The use of analgesics and antidepressants such as amitryptiline, duloxetine and codeine may decrease physical discomfort and improve mood but need careful monitoring as they may worsen fatigue and lead to poor concentration. Adverse reactions to certain anaesthetic agents are well-documented in PPS. Post-anaesthesia fatigue, somnolence and weakness are well recognised, and fatal outcomes due to respiratory arrest have also been reported [200, 201]. The diagnosis of PPS needs to be carefully discussed with the anaesthesiologists, so the appropriate muscle relaxants and anaesthetics can be used, and patients should be advised of the possibility of a prolonged post-operative phase [202].

1.4 Conclusions

Despite being one of the most devastating neurodegenerative conditions in the world, surprisingly limited research is undertaken in post-polio syndrome. Its pathogenesis remains elusive, no sensitive diagnostic tools have been developed, and validated prognostic and monitoring markers are lacking. Non-motor symptoms of PPS have considerable quality of life implications and are notoriously challenging to manage. The aetiology of fatigue in PPS is yet to be elucidated and successful individualised management strategies are needed to maintain mobility, independence and patient autonomy. There is striking a paucity of neuroimaging studies in PPS that could provide anatomical insights into the substrate of extra-motor symptoms. Ultimately, the characterisation of PPS-associated pathology may help research efforts in other motor neuron disease.

Author(s) and year of publication	Study Design /selection criteria of PPS patients	Number of follow-up time points	Follow-up interval (months)	Number of participants receiving drug / placebo	Assessment tools used	Key study findings
Prednisone						
Dinsmore et al. 1995 [183]	RDBPC/U	3	3	7/7	MRC scale, MVIC using electronic strain gauge tensiometer, fatigue on a 0-3 scale	– Short-lived improvement in muscle strength – No improvement in fatigue – Not recommended
Amantadine						
Stein et al. 1995 [184]	RDBPC/ S (fatigue)	2	2	10/13	FSS, VAS-F, MMPI, BDI, somatization scale, reaction time evaluation	– Not superior to placebo for fatigue
Pyridostigmine						
Trojan et al. 1999 [187]	RDBPC/S(fatigue/muscle weakness)	6	at 6 weeks, 10 weeks and 6 months	43/42	SF-36, modified TQNE, MVIC by electronic strain gauge, Hare Fatigue Symptom Scale, FSS, IGF-1 serum levels	– Very weak muscles became slightly stronger – IGF-1 increased in compliant patients – No clear benefits on QoL, muscle strength and fatigue

Horemans et al. 2003 [188]	RDBPC/S (fatigue and muscle weakness)	5	0.75	31/31	NHP, FSS, 2MWT at comfortable pace, time to walk 75m at fastest speed, ambulatory activity monitor, MVC by chair dynamometer, MVA by interpolated stimulation; muscle fatigability by sEMG during 30s sustained isometric contraction at 40% of MVC, NMJ defects by jitter on S-SFEMG	– No significant effects on fatigue – Significant effects on walking distance Little effects on walking duration, muscle strength, MVA – Limited benefits in physical performance
Modafinil						
Chan et al. 2006 [186]	RDBPC cross-over/ S (fatigue)	12	0.25	7/7 Cross-over 7/7	PFS, ESS, aural digit spans, reaction time	– Not effective in fatigue
Vasconcelos et al. 2007 [185]	RDBPC cross-over/ S (fatigue)	2	1.5	18/18 Cross-over 18/15	FSS, VAS-F, FIS; SF-36	– Not superior to placebo in fatigue and QoL improvement
Co-enzyme Q10						
Skough et al. 2008 [189]	Parallel RDBPC/S(ability to perform resistance training)	2	3	7/7	Sit-stand-sit test (SSS); Timed up & go (TUG) test, 6MWT, dynamometer, bloods for CK, LD	– No change in CK or LD – No additional effects of the Co-enzyme Q10 supplementation during resistance training
Peel et al. 2015 [190]	Parallel RDBPC/S (fatigue)	2	2	54/49	MAF (revised Piper Fatigue Scale), FSS	– Not effective in fatigue

Lamotrigine						
On et al. 2005 [191]	RDBPC/S (ambulatory with lower limb involvement only)	3	0.5	15/15	VAS, NHP, FSS	– Superior to placebo for pain, fatigue and QoL as detected in VAS, NHP, FSS
Intravenous immunoglobulin (IVIg)						
Gonzalez et al. 2004 [38]	Controlled open-label/U	2	1.5-2	16PPS; 26OND/0	CSF for CSF-MC, PB for PBMC, real-time quantitative RT-PCR for relative quantitation of mRNA	– Significant decrease of CSF-MC expression of TNF-α and IFN-γ not seen in PBMC expression of cytokines
Kaponides et al. 2006 [192]	Uncontrolled open-label/S (ambulatory, BMI<28)	3	at 2 and 6 months	14/0	Dynamic dynamometer, 6MWT, SF-36	– No significant effect on muscle strength and physical performance
Gonzalez et al. 2006 [197]	RDBPC/U	2	3	67/68	Dynamometer, SF-36, 6MWT, TUG, PASE, sway, sleep quality, VAS, MFI-20	– Positive changes in muscle strength, physical activity and those with significant pain – No change on QoL, fatigue sleep quality, 'better' limb muscles or mild pain
Farbu et al. 2007 [40]	RDBPC/U	5	3	10/10	MAF (revised Piper Fatigue Scale), FSS, CSF and PB for expression of cytokines (TNF-α, IFN-γ, IL-6, IL-1β, IFN-β, IL-10) using ELISA	– Positive effects on pain after 3 months – No effects on muscle strength and fatigue

						– TNF-α increased in CSF
Werhagen et al. 2011 [194]	Uncontrolled open-label/S (pain)	2	6	45/0	Neurological examination, sensory testing, soft tissue palpation and joint assessment, VAS, pain classified according to IASP	– Better results on pain in younger, those with more pronounced paresis, had acute polio <10yo
Östlund et al. 2012[195]	Uncontrolled open-label/S(fatigue, muscle weakness)	2	6	113/0	SF-36, PASE, VAS	– Likely responders include those with pain intensity above VAS of 20mm, younger than 65yo and paresis in lower extremities
Gonzalez et al. 2012 [65]	RDBPC and controlled quantitative cytokine study/U	2	12	CSE: 20/21 CAS: 20/30	CSE: SF-36, 6MWT, VAS CAS: CSF and PB for cytokines (TNF, IL-23, IFN-γ, TGF-β, IL-10, IL-13) using RT-PCR	– Improvement in QoL but not in pain and walking ability compared to placebo – Decline in CSF IFN-γ and IL-23, TNF and increase in IL-10 and IL-13 – No changes in PB cytokine levels
Bertolasi et al. 2013 [193]	RDBPC/U	3	2	24/26	SF-36, MRC scale, dynamometer, 6MWT, VAS, 101-PNR, FSS	– Improvement in QoL; mental activity subscale – no effects on gait, muscle strength, fatigue and pain
L-Citrulline						
Schmidt et al. 2017 [198]	RDBPC/U	5	6	15/15	6MWT, MFM scale, qMRI, MRS, bloods for muscle necrosis (CK), oxidative stress (8OHDG, 4-HNE), nitrosative stress(nitrotyrosine, cGMP), mitochondrial-related	– Ongoing clinical trial

					genes (*Citratsynthase, Cytochrome C oxidase subunit 1, Succinate dehydrogenase subunit A)*, QMT using HHD, SIPP,IBM-FRS,WHOQOL-BREF	
Respiratory support						
Kaminska et al. 2015 [164]	Feasibility/S (restrictive respiratory defects)	2	3	7ALS, 7PPS, 5MD	SF-36, SIP, standard spirometry (FVC, FVC% predicted, LIC, LIC-FVC difference, PCF, MIP, MEP)	– LVR Feasible – Encouraging effects on respiratory mechanics – LIC increased
Gillis-Haegerstrand et al. 2006 [165]	Randomized comparative/S (using VCV)	2	30 min	8	BP, oxygen saturation, ABG, indirect calorimetry (SaO_2, VO_2, VCO_2, REE, RQ, RR, IPAP)	– BiPAP PSV decreases oxygen cost of breathing in PPS with respiratory failure without decreasing ventilation efficiency. – Significant $PaCO_2$ decrease using this ventilation modality. – Maintains adequate ventilation in PPS patient with resp. failure
Barle et al. 2005 [167]	Comparative /S (nocturnal invasive CMV)	7	30 min	9	BP, oxygen saturation, ABG, indirect calorimetry (SaO_2, VO_2, VCO_2, REE, RQ, MV,RR, IPAP)	– Invasive BiPAP reduces oxygen cost of breathing in long-standing tracheotomized PPS compared to CMV.

Exercise program						
Murray et al. 2017 [149]	Assessor blinded rCT/U	2	2 months	26/29	6-MAT, PASIPD, 6MWT, FSS, SF-MPQ-2, QMA, exercise log	– Home-based ergometry is a well-tolerated form of aerobic exercise – No improvement of physical fitness, fatigue, activity – Slight decrease in BP in interventional group
Pramipexole						
Kumru et al. 2014 [93]	Uncontrolled open label/U	3	At 0, 2months and 6 months	16/0	RLS severity scale	– Significant decrease of RLS severity detected on RLS rating scale – Maintenance of improvement of RLS with pramipexole at 6 months follow-up

Table 1- 1 Pharmaceutical and non-pharmaceutical clinical trials in post-polio syndrome study characteristics and key outcomes.

2 Imaging in Motor Neuron Diseases (MNDs)

2.1 Clinical considerations in Motor Neurone Disease

2.1.1 Diagnostic considerations

The early diagnosis of motor neurone disease (MND) can be challenging and it often takes a number of visits to various specialists to establish the diagnosis. Early symptoms of MND are often very subtle, intermittent and non-specific and may therefore be attributed to fatigue or mistaken for other conditions. Intermittent falls, clumsy hands, slurred speech after a long day, muscle twitching and coughing fits are not uncommon many months before the possibility of the diagnosis is even raised.

The first point of contact is often the GP, who typically performs a thorough physical examination. Sometimes, no objective clinical findings can be identified early in the course of the disease. The average interval between the first symptoms and the definite diagnosis is around twelve months. The protracted diagnostic journey in MND is not a reflection on a specific healthcare system, geographical region, GP or neurologist, rather the inherent nature of any slowly progressive neurodegenerative condition.

Compared to other neurological conditions, MND exhibits considerable variability in initial symptoms, disease progression rates and age of onset. It is not uncommon for MND-associated symptoms to be initially attributed to other conditions, such as carpal tunnel syndrome, slipped disks, and peripheral nerve problems, or even be mistaken for psychological issues at first. It is easy to criticise misdiagnoses in retrospect when the manifestations of MND are obvious, but as it is a rare disease, the initial consideration of more common conditions may be reasonable.

2.1.2 What happens after referral?

Once a referral is made to a neurologist, a systematic and structured approach is needed to confirm a suspected diagnosis as follows:

- a detailed clinical history

- family history
- medication history
- occupational history
- exposure to environmental factors
- physical and neurological examination.

These are all indispensable. Electrical, laboratory and radiological tests are not necessarily required to confirm the diagnosis of MND, but these are often undertaken to rule out alternative neurological diagnoses which may mimic MND. The specific tests recommended by the neurologist depend on the findings on examination and the constellation of initial symptoms.

2.1.3 Ruling out alternative diagnoses

Brain and spinal cord scans (MRI) are often helpful to rule out alternative structural or inflammatory processes, but they are not absolutely necessary.

- Patients with bulbar-onset disease are particularly likely to benefit from brain imaging.
- Spinal MRI is useful to rule out cord compression, tumours, vascular malformations, inflammatory changes, or the compression of spinal nerve roots. As many people suffer from some degree of back pain and protruding disks, clinical and radiological findings need to be carefully integrated. Depending on the initial symptoms, additional tests, such as blood tests and cerebrospinal fluid analyses from a spinal tap may also be performed. The main role of these tests in suspected motor neurone disease is to rule out alternative diagnoses.
- Blood tests are useful to assess for systematic infections, autoimmune conditions, endocrine abnormalities, vitamin deficiencies, and malignancies.
- Spinal fluid analyses can also be helpful to reassuringly rule out central nervous system infections, malignancy or autoimmune conditions. Spinal taps are typically performed as an outpatient or day hospital procedure, sometimes under radiological (X-ray) guidance.

- Genetic testing is not required to establish the diagnosis of MND but can be helpful to confirm specific MND types such as Kennedy's disease (SBMA).

2.1.4 Confirming the diagnosis

'Electrical' tests (formally called 'neurophysiology' or 'electrophysiology' studies) are particularly helpful to evaluate affected muscles and nerves. These tests look at spontaneous electrical activity in the muscles suggestive of motor nerve degeneration.

Voluntary muscle activity is controlled by a two-tier command structure. The upper motor neurones (UMN) are located in the area of the brain called the 'motor cortex'. These neurones have very long tails (axons) that descend through a convoluted path into the brainstem and spinal cord. They interface with the lower motor neurones (LMN) that leave the spinal cord and make their way to specific muscles.

The assessment of the *upper motor neurone system* requires a very careful physical examination, focusing on muscle tone and various reflexes. Upper motor neurone signs include brisk reflexes, cramping, and muscle stiffness. The clinical manifestations of *lower motor neurone degeneration* include muscle wasting, twitching, and weakness. To establish the diagnosis of amyotrophic lateral sclerosis (ALS), one has to demonstrate that both the upper and lower motor neurone systems are affected. Neurologists typically distinguish between limb onset ('spinal-onset') and voice or swallowing onset ('bulbar-onset') forms of ALS.

While MND can be diagnosed by any neurologist, it is often helpful to see a neurologist with a special interest in neuromuscular disorders or someone who cares for many patients with MND. A second opinion is often sought, and there are cases when the diagnosis is suspected for a long time, but formal electrophysiological or clinical criteria are not met.

2.1.5 Manifestations of the Disease

Bulbar manifestations of MND include slurred speech, coughing fits when eating or drinking, difficulty with crumbly foods, and tongue wasting. An unusual manifestation of the disease is what is called 'pseudobulbar affect' (PBA) or 'pathological crying and laughing' (PCL). This refers to uncontrollable crying or laughing, which is a distressing

symptom. Patients with PBA may start tearing up watching an emotive TV commercial or start laughing excessively in situations which they don't find particularly amusing. These symptoms may cause embarrassment and may limit a person's social activities and lead to avoidance of public places. The recognition and discussion of PBA is very important as there are a number of medications which can bring these symptoms under control.

It cannot be emphasised enough that muscle twitching, slurred speech, muscle wasting, muscle stiffness can be caused by *many other medical and neurological conditions*, so the constellation of these symptoms does not necessarily suggest MND.

It is also noteworthy that the term 'MND' encompasses a multitude of syndromes which include:

- amyotrophic lateral sclerosis (ALS)
- Kennedy's disease (spinal and bulbar muscular atrophy: SBMA)
- primary lateral sclerosis (PLS)
- progressive muscular atrophy (PMA)
- spinal muscular atrophy (SMA)
- rare conditions such as Mills' syndrome

Primary lateral sclerosis (PLS) is associated with selective *upper motor neurone* (UMN) dysfunction, and people with PLS typically experience falls, lower limb stiffness and cramping. It carries a relatively good prognosis. It is however a particularly challenging diagnosis to establish because ALS can also present with predominant upper motor neurone signs initially.

Kennedy's disease is a genetic disorder involving the *lower motor neurone* system. It affects male patients and has a number of non-neurological manifestations in addition to weakness, muscle twitching and wasting.

It is important to carefully distinguish all these syndromes from ALS, because they are associated with different clinical outcomes and care needs. The medical terminology may be somewhat confusing, but the main message is that there are many types of motor

neurone disease, each associated with a distinct set of symptoms, medical outcomes, and specific management needs. Motor neurone disease is merely an umbrella term and patients with MND have markedly different symptoms and care needs.

2.1.6 The multidisciplinary team

Once the diagnosis of MND is established, a thorough multidisciplinary assessment is required. Multidisciplinary MND clinics have been carefully set up to pre-empt, monitor and manage MND-associated symptoms. In MND care, a multidisciplinary team (MDT) typically consists of the following:

- an experienced MND specialist nurse
- a social worker
- a dietician
- a speech and language therapist
- an occupational therapist
- a physiotherapist
- a neuropsychologist
- a neurologist
- a neurophysiologist/electrophysiologist
- a respiratory physician
- an interventional radiologist for feeding tube insertions.

There is ample evidence that specialist MDT clinics provide effective, individualised management for a variety of presentations and disease variants. Given the relative rarity of MND compared to other neurological conditions, the contribution of experienced healthcare professionals to MND clinics is invaluable. The role of the multidisciplinary team is twofold: (1) screening for symptoms which may be associated with the disease and (2) the dynamic management of emerging symptoms.

2.1.7 Initial assessment

The initial MDT assessment would typically include a comprehensive evaluation for nutritional status, swallowing, speech, mobility, dexterity, muscle stiffness, respiratory function (breathing), cognition, and social support.

- Breathing can be assessed by pulmonary function tests (PFTs) or portable devices such as the sniff nasal inspiratory pressure (SNIP) testing.
- Overnight oxygen levels can be monitored by a finger-clipped device called a pulse oximeter, which helps to identify dips in oxygen levels non-invasively.
- Nutritional status, diet, weight loss are carefully monitored and patients with swallowing difficulties may need feeding tube placement to maintain their weight. The optimal timing of feeding tube insertion requires experience and careful coordination between multidisciplinary team members.

2.1.8 Supportive interventions for independence

The governing concept behind any supportive intervention in MND is to maintain independence and adapt to existing lifestyles, while maintaining dignity and autonomy. Dietetic, respiratory and mobility interventions are tailored to individual care needs, so they can be implemented in the patient's community, home environment, during travel or at work.

Maintaining mobility and reducing fall risk is an important aspect of preserving independence. While some patients suffer from weak ankles ('foot drop'), wrists ('wrist drop') or neck ('head drop'), others suffer from considerable stiffness in their limbs and joints. Depending on individual symptom profiles, personalised assistive devices such as walking aids, ankle-foot orthoses (AFOs), wrist splints, wheelchairs, chin lifts, or stair lifts help to assure safety at home and prevent falls.

2.1.9 Monitoring respiration

Another key aspect of MND care revolves around the careful monitoring for respiratory insufficiency. Breathing problems may present as:

- early-morning headaches
- exercise intolerance
- unexplained fatigue

- recurrent chest infections
- difficulty clearing secretions.

Respiratory management typically centres on the introduction of non-invasive ventilation (NIV) which can be used overnight, or during the day as needed. These machines provide a pre-set pressure via a facial mask to help breathing and synchronise with the patient's natural respiratory cycles. It often takes some time to get used to these machines, but most patients tolerate them well and report significantly improved energy levels. These machines are relatively light and portable.

2.1.10 Managing fatigue

Fatigue is a common symptom of MND, which is likely to stem from a combination of factors, owing to respiratory compromise, medications, nutritional deficits, poor sleep, discomfort, low mood and possibly disease-specific metabolic changes. It is important to carefully review these factors and adjust medications if necessary to reduce fatigue. Sleep is often fragmented in ALS, something that also needs to be discussed with the MND multidisciplinary team. Discomfort from muscle aches, pain in pressure areas, difficulty synchronising with non-invasive ventilation, and medications may all contribute to poor sleep in MND. All of these factors can be improved once the main cause of insomnia is identified. Palliative care interventions are useful at any stage of the disease as they improve quality of life and reduce discomfort while keeping individual preferences in mind.

2.1.11 Speech and swallowing

In patients with speech and swallowing difficulties (bulbar impairment), a sore and coated tongue is not uncommon. This is typically caused by the reduced natural movement of the tongue combined with deeper grooves secondary to wasting, which makes it prone to a superficial fungal infection called 'oral candidiasis'. This is important to recognise as it can be effectively treated with anti-fungal medications. Drooling or excessive salivation is a distressing, and relatively common symptom of the bulbar form of the disease, which can also be treated efficiently.

Patients with feeding tubes can often continue to enjoy food and drinks in the usual way. Feeding tubes help to maintain weight by delivering sufficient calories, and are often put in place relatively early in the course of the disease. Feeding tubes are concealed under the clothing so they are not visible and they don't impede walking or driving. Feeding tubes are either referred to as RIG (radiologically inserted gastrostomy) or PEG (percutaneous endoscopic gastrostomy) tubes depending on the mode of insertion. These tubes don't typically interfere with lifestyle, as the dietician can either set up intermittent or continuous overnight feeds depending on individual preferences.

2.1.12 Therapy and research

While the mainstay of MND care is a series of supportive multidisciplinary interventions, the medication riluzole offers survival benefits and is typically very well tolerated. Some nausea and mild gastrointestinal side-effects are sometimes reported, but temporary dose-reduction is often sufficient to improve the symptoms. Another medication called edaravone has been approved in the US but clinical trials in Europe are ongoing to evaluate its therapeutic benefits. A number of other promising clinical trials are underway and recent therapeutic breakthroughs in other neuromuscular conditions, such as spinal muscular atrophy, give cause for optimism for the development of novel drugs in MND.

Research in MND can be categorised into distinct areas, all directly relevant to the development of new therapies. Any research study undertaken in MND is strictly regulated by local ethics committees, international consortia and is governed by European data protection laws. People with MND are asked to provide consent before study participation and are informed how data will be stored, coded and utilised in the future. The main objectives of research in MND are:

- to develop efficient medications
- gain a better understanding of how the disease spreads
- explore the factors behind the considerable variability in the disease
- develop better diagnostic and monitoring tools.

2.1.13 Genetic studies

Genetic studies focus on the understanding of genetic susceptibility for MND, and deciphering the causes of the disease. While some patients have family members with MND (familial cases), the majority of MND patients are unaware of affected family members (sporadic cases). Genetic studies rely on blood samples from both patients and controls; that is why patients are approached to give a blood sample if they consent to genetic research. The blood is carefully stored and processed, and DNA is extracted in dedicated laboratories. The discovery of novel genetic mutations is an exciting, yet labour intensive process, which often requires large international collaborations.

2.1.14 Epidemiology studies

Epidemiology studies seek to understand gene–environment interactions and the role of lifestyle factors (exercise, sport, smoking, diet), environmental exposures (pollution, radiation), and family history in the pathogenesis of ALS. Data for epidemiology studies are typically collected through carefully designed questionnaires and are either mailed to the patients or administered through semi-structured interviews. The analysis of these data requires considerable care, and complex mathematical models are used either at a population level or in collaboration with other centres. For the accurate recording of disease incidence, potential environmental risks, age of onset, lifestyle and geographical location, national MND registries have been set up to collect, store and analyse data.

2.1.15 Biomarker studies

Biomarker studies seek to identify objective, quantifiable markers to aid early diagnosis, accurate monitoring in pharmacological trials and the development of reliable prognostic indicators. There are two main themes in biomarker research; these are colloquially called ‘wet’ and ‘dry’ markers. Research into ‘wet’ biomarkers focuses on the measurements of various proteins, hormones, markers of inflammation and indicators of cell damage in the blood and spinal fluid. ‘Dry’ biomarkers focus on the detection and monitoring of MND-associated pathology using non-invasive techniques such as neuroimaging

2.1.16 Neuroimaging

Specially tuned scanners can generate high-resolution images of the brain and spinal cord and reliably detect brain changes responsible for specific symptoms. This type of research has already helped us to decipher which brain regions are particularly vulnerable in MND and which brain regions remain relatively resilient to the disease.

The large datasets generated by MRI scanners can be analysed to pick up subtle changes over time and help us to understand how the disease spreads from one region to another. MRI studies have shown how the internal wiring of the brain (referred to as the white matter) gets selectively affected and how the cortical part of the brain (grey matter) shows focal degeneration leading to specific disability patterns. Other techniques, such as electroencephalography (EEG) or magnetoencephalography (MEG) can detect electrical oscillations from the brain and characterise the dysfunction of specific neural networks. These studies are also non-invasive and are generally well tolerated by the patients.

2.1.17 Neuropsychology studies

Neuropsychology studies investigate changes in thinking, decision making, memory and language. The characterisation of these changes is important for improved clinical trial designs and compliance with supportive interventions. While neurologists often perform simple screening tests, expert neuropsychologists can detect subtle changes. Neuropsychological tests typically include pen and paper tests and questionnaires, but they can also be administered to patients who have difficulty speaking or writing. Performance on these tests needs to be carefully interpreted using reference values from age-matched controls, therefore no instant feedback is typically given.

2.1.18 Promising international research

Most MND teams are active members of the international MND research community and meet several times a year to exchange information about emerging therapies, new discoveries and breakthrough research findings. Research in MND has gained unprecedented momentum in recent years; the number of attendees at international meetings has grown exponentially. Awareness of MND has increased considerably thanks to the tireless work of charities, patient advocacy groups and caregivers.

In light of the momentous academic advances and the record number of clinical trials in 2021, the future of MND research looks promising.

2.2 The imaging signature of hexanucleotide repeat expansions in *C9orf72*

2.2.1 Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are progressive neurodegenerative conditions with overlapping clinical, genetic, pathological and imaging features [203, 204]. ALS is predominantly characterised by upper and lower motor neuron degeneration but is also associated with extra-motor involvement [205, 206]. Frontotemporal dementias encompass a heterogeneous group of conditions characterised by progressive frontal and temporal lobe atrophy manifesting in a spectrum of cognitive, behavioural and langue dysfunction. About 30% of patients with ALS and up to 15% of FTD patients experience an overlap between the two syndromes [207, 208]. The clinical overlap between the two conditions has been observed for decades and has been further elucidated by the discovery of hexanucleotide GGGGCC repeat expansion in *C9orf72* in 2011 [209, 210]. The GGGGCC repeat expansion in *C9orf72* gene is thought to cause up to 40% of familial ALS and FTD [210] and approximately 4-7% of sporadic cases in European and North American populations [211, 212]. The clinical manifestations of the genotype show striking heterogeneity with regards to the clinical phenotype, age of onset, survival, lower motor neuron involvement, rate of disease progression etc. [213-215]. The most common phenotypes associated with *C9orf72* are behavioural variant FTD (bvFTD), ALS and comorbid ALS-FTD [216, 217]. The considerable differences in clinical manifestations make it difficult to describe unifying imaging signatures, as small sample sizes are typically combined with preferential recruitment streams from either FTD or ALS centres [218]. With the advent of antisense oligonucleotide (ASO) therapies the meticulous characterisation of *C9orf72*-associated changes is particularly timely as the optimal timing for therapeutic interventions is not yet established. Presymptomatic studies suggest that repeat carriers already exhibit widespread degenerative changes in their thirties [219, 220] suggesting the early intervention may be particularly beneficial [221]. Some authors suggest that neurodevelopmental factors should be considered in ALS [222], especially in association with *C9orf72* [220]. The identification of markers to predict phenoconversion and age of symptom onset has important practical relevance [223]. Additionally, with the emergence of genotype-specific therapies, best practice recommendations are urgently needed for the genetic screening of all symptomatic patients and genetic counselling of relatives of symptomatic mutation carriers [221].

Accordingly, the aim of this review is to systematically review the current literature of *C9orf72-associated* neuroimaging features, identify common study limitations and highlight the contribution of individual studies. The overarching objective of this review is to outline a desirable approach for future *C9orf72* studies to address the practical biomarker requirements for impending ASO studies.

2.2.2 Methods

A formal literature review was conducted on PubMed in accordance with the Strobe guidelines. The search term "*C9orf72*" was paired individually with the following keywords: "amyotrophic lateral sclerosis", "motor neuron disease", "frontotemporal dementia", "frontotemporal lobar degeneration", "ALS-FTD", "neuroimaging", "magnetic resonance imaging", "diffusion tensor imaging", "functional MRI", "single photon emission computed tomography", "positron emission tomography", "presymptomatic", "asymptomatic", "post-mortem". Selection criteria included original research articles pertaining to neuroimaging studies in *C9orf72*. Only articles in English were selected for review. Opinion pieces, previous review articles, case reports, *C9orf72* research studies without imaging were not reviewed. The selected articles were systematically reviewed for sample size, control groups, recruitment strategy, study design, imaging methodology, statistical models, genetic screening methods, clinical assessments, and the availability of post-mortem pathological validation.

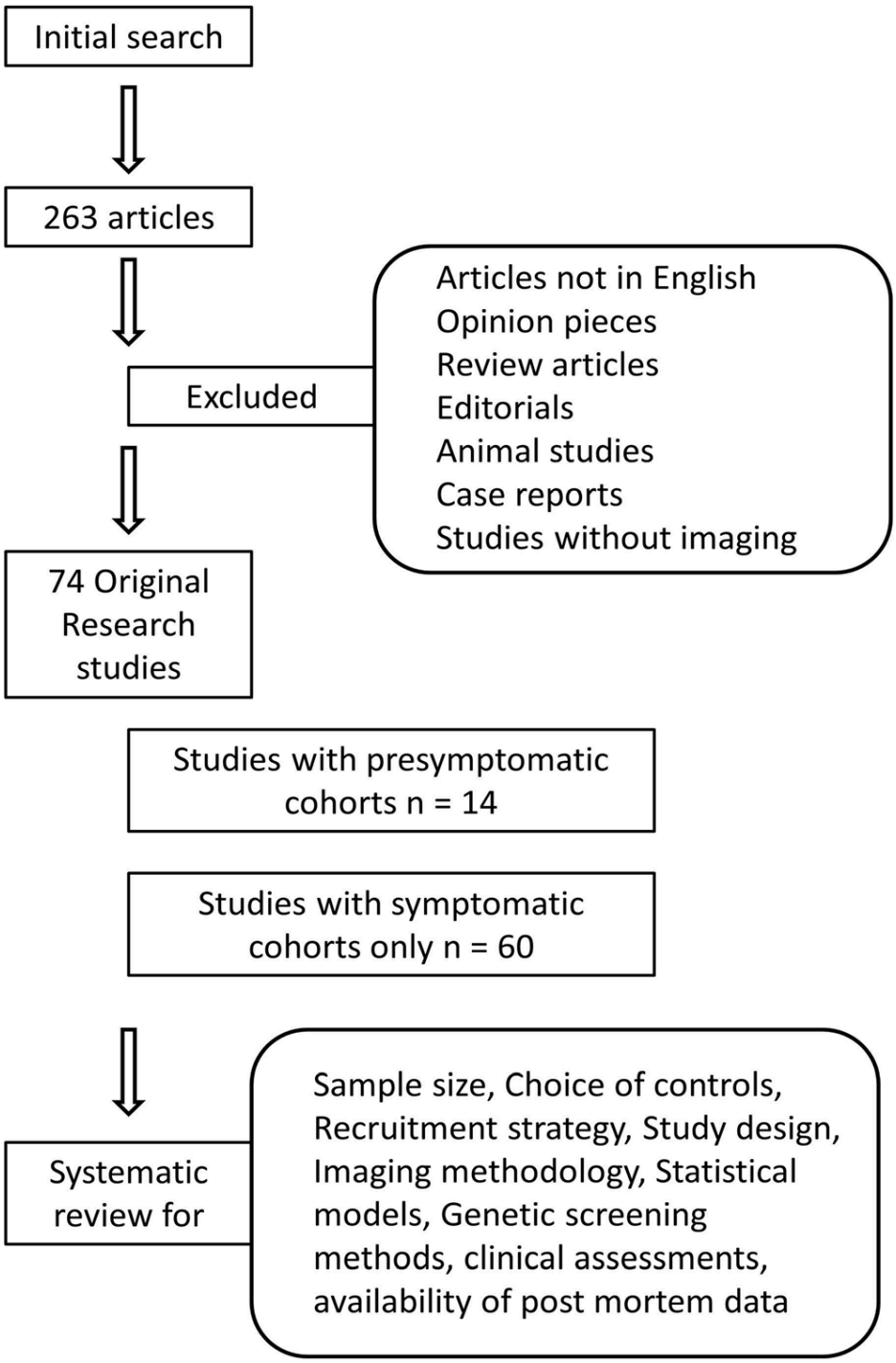

Figure 2- 1 Systematic review flow chart

2.2.3 Results

Based on our initial search, 263 articles were identified, 74 of which met our core review criteria for systematic analysis. (**Figure 2-1**)

2.2.3.1 Neuroimaging findings in ALS associated with C9orf72

C9+ALS is associated with diffuse symmetrical cortical volume loss [206, 214, 224-227] involving the primary motor areas, but also extending beyond motor regions to include orbitofrontal, opercular, fusiform, lingual, cingulate and temporal regions when contrasted to C9-ALS [214, 224, 226, 228]. C9+ALS patients are thought to have less cortical thinning in the motor cortex and greater atrophy in extra-motor regions, particularly in posterior areas, such as the occipital cortex and cuneus compared to C9-ALS patients [205, 224, 226]. On longitudinal assessment, no progressive cortical thinning could be detected over a 6 months follow-up period, however, ventricular expansion has been linked to cognitive decline [224]. Subcortical grey matter atrophy is a consistent radiological finding preferentially affecting the thalamus [224, 226, 229], but also the hippocampi, amygdala, nuclei accumbens, caudate, putamen and striatum [230-232]. While considered more extensive in C9+ ALS [228, 230], extensive subcortical grey matter degeneration can also be identified in C9-ALS and C9- PLS [225, 232-235]. The dysfunction of frontostriatal networks are likely to contribute to the unique neuropsychological profile of C9+ALS encompassing executive dysfunction, apathy and impairments in social cognition [230]. A recent study [236] highlighted the selective involvement of thalamic nuclei in C9+ ALS preferentially affecting the mediodorsal-parateniual-reuniens group of nuclei, which play an important role in executive functioning. This is in contrast to C9+FTD studies, where preferential pulvinar atrophy is thought to be a hallmark feature of C9+FTD [237]. Amygdala atrophy is thought to be a feature of *C9orf72* ALS with the preferential involvement of the lateral nucleus and the cortico-amygdaloid transition in GGGGCC hexanucleotide carriers [238, 239]. Cerebellar degeneration was noted in association with C9+ALS by some, [226, 229] but this is not a consistent finding [225], and cerebellar pathology can also be readily detected in C9-ALS [240]. Hypothalamic volume reductions were also identified in admixed ALS mutation carriers, including C9+ patients [241]. Despite evidence of faster respiratory decline in *C9orf72*, no preferential brainstem atrophy was identified cross-sectionally or longitudinally on imaging [242-244].

On quantitative cord imaging, segmental C2 and C3 atrophy was identified in C9+ ALS patients without longitudinal changes over an average of 5.4-month follow-up interval [245]. Diffusion tensor imaging (DTI) studies suggest that C9+ALS patients have more widespread white matter abnormalities including the genu of corpus callosum, anterior commissure, and intrahemispheric association tracts including the uncinate, inferior longitudinal and superior longitudinal fasciculi [218, 225-228, 230]. On longitudinal assessment, progressive DTI alterations can be readily detected within a 6-month follow-up period and an anterior to posterior propagation pattern has been suggested [218]. Diffusion metrics of the frontal white matter in C9+ALS have been correlated to the ALSFRS-r, cognitive and behavioural scores [218]. On rs-fMRI, C9+ALS exhibits reduced SMN functional connectivity compared to C9-ALS [226]. The PET literature of C9+ALS is surprisingly limited [57]. C9+ALS patients typically exhibit decreased glucose metabolism in peri-rolandic and prefrontal areas [246] and interestingly, hypermetabolism has been reported in the same regions in the presymptomatic stage [247]. In contrast to C9-ALS, C9+ALS patients show widespread cerebral hypometabolism including the anterior and posterior cingulate, insula, caudate, thalamus as well as frontal and superior temporal cortical regions [246, 248]. Hypermetabolism was reported in the midbrain, bilateral occipital cortex, globus pallidus and inferior temporal cortex [248, 249]. Increased [18F]-AV1451 uptake was also observed in the entorhinal cortex of C9+ALS compared to healthy controls [250]. Compared to various MRI applications and PET, EEG and MEG approaches seem to be underutilised despite the important functional insights offered by these modalities in ALS and FTD [251-257].

2.2.3.2 Neuroimaging findings in ALS-FTD associated with C9orf72

On structural MRI, C9+ALS-FTD patients consistently show widespread extra-motor pathology in orbitofrontal, cingulate and opercular regions [206]. Motor cortex findings in C9+ALS-FTD are more inconsistent due to the clinical heterogeneity of this cohort from a motor perspective [224]. While C9- and C9+ ALS-FTD patients exhibit overlapping patterns of atrophy, C9+ patients tend to display greater dorsal frontal, posterior cortical and cerebellar atrophy compared to C9- ALS-FTD patients [216]. Considerable thalamic atrophy is a consistent finding of C9+ALS-FTD patients both compared to healthy controls and C9-ALS-FTD [216, 237]. C9+ ALS-FTD patients are thought to have preferential subcortical pathology in regions connected to motor and

sensory cortical areas, while C9- ALS-FTD patients exhibit thalamic and striatal pathology in areas projecting to rostral-motor and executive cortical areas [258].

2.2.3.3 Neuroimaging findings in FTD associated with C9orf72

The consensus observation of C9+ FTD studies is a relatively symmetrical pattern of frontal, temporal, insular and cingulate atrophy with marked posterior involvement encompassing the precuneus, parietal regions and occipital lobes [215, 259-264]. In comparison to C9-FTD, C9+FTD is thought to preferentially involve posterior cerebral regions such as the parietal lobes [216, 265], the precuneus [266] and is less likely to affect regions primarily associated with bvFTD such as the orbitofrontal cortex, anterior cingulate, insula and temporal poles [267, 268]. Subcortical volume loss has also consistently been reported in C9+FTD, thalamic atrophy in particular [216, 260, 261, 263-266, 269]. While thalamic atrophy can also be found in other genetic variants of FTD [237, 270], medial pulvinar thalamic nuclei degeneration is thought to be unique to *C9orf72* [237, 266]. Cerebellar degeneration has also been reported in *C9orf72* compared to both healthy individuals [259-261, 264] and C9-FTD [265]. C9+ FTD is proposed to be associated with the smallest global cerebellar volume among genetic variants of FTD [271], and specifically linked to lobule VIIa-Crus I degeneration in the superior-posterior cerebellum [271]. Basal forebrain [272], amygdala, basal ganglia, striatum and hippocampal atrophy are also commonly observed in C9+FTD [264, 266, 267]. The CA4, CA1 hippocampal subfields and dentate gyrus are preferentially affected in *C9orf72* [273]. Amygdalar nuclei were also specifically investigated. The superficial group of nuclei (central nucleus, cortical nucleus, medial nucleus, anterior amygdaloid area) and accessory basal nucleus exhibits selective involvement in *C9orf72* [274]. A trend of hypothalamic atrophy was also observed in C9+ FTD driven by the degeneration of anterior superior and tuberal hypothalamic regions [275]. Diffusion tensor imaging (DTI) in C9+FTD has consistently captured symmetrical white matter alterations in commissural, corticospinal, cerebellar and frontal tracts [260, 261, 266]. Functional connectivity patterns were also assessed extensively in *C9orf72* using resting-state functional MRI (rs-fMRI). In C9+ FTD, connectivity alterations in the salience network (SN) are associated with neuropsychiatric manifestations [266]. Increased attention network (AN) connectivity was also noted in C9+bvFTD and linked to neuropsychiatric manifestations [276]. Longitudinal studies captured progressive cortical atrophy,

ventricular expansion, FA decline in the cingulum and increasing MD in the uncinate [260, 268, 277]. On metabolic imaging, C9+ FTD exhibits symmetrical frontotemporal hypometabolism [215], while the C9+ semantic variant FTD is associated with right temporal polar involvement [249]. Furthermore, C9+FTD patients show decreased glucose metabolism in the thalami compared to C9-FTD further supporting the preferential involvement of the thalamus in *C9orf72* [278]. Another C9+FTD study captured additional striatal, premotor and motor involvement [279]. Besides FDG-PET, other ligands were also investigated in C9+FTD such as [18F]-AV-1451 which showed increased binding in left fusiform and temporal lobe in C9+FTD [280]. An 18-F-Flortaucipir study identified reduced uptake in inferior frontal lobes of C9+bvFTD [281].

2.2.3.4 Presymptomatic radiological changes in C9orf72

Considerable cerebral changes have been consistently detected in asymptomatic hexanucleotide carriers [282, 283]. On structural MRI, frontal [284], temporal [285], parietal [286], insular [285, 287], cingulate [264], precuneus [219] and occipital [288] changes have been described, sometimes with the sparing of primary motor [231, 262] and sensorimotor regions [219]. Abnormally low gyrification index has been observed in asymptomatic *C9orf72* mutation carriers in the anterior cingulate, left precentral gyrus, right inferior parietal lobule and right superior occipital gyrus seen in patients in their early 30s [289]. Early cerebellar involvement has been identified by some studies [264, 285-287] but not confirmed by others [247, 284]. Thalamic atrophy [219, 264, 285-288, 290, 291] has also been consistently reported along with the involvement of other subcortical grey matter structures such as the caudate [231, 284], putamen [231] and striatum [290]. On diffusion tensor imaging (DTI), asymptomatic GGGGCC hexanucleotide carriers displayed white matter integrity changes in a multitude of association, projection and commissural fibres. The presymptomatic white matter signature of *C9orf72* includes pathological changes in the forceps minor, inferior fronto-occipital fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, anterior and posterior thalamic radiations, corpus callosum, cingulum and corticospinal tracts [219, 286, 287, 290, 292]. While white matter changes are traditionally appraised by DTI-derived metrics, NODDI is thought to be more sensitive in capturing early white matter pathology, and neurite density measures are increasingly applied to ALS genotype studies, including presymptomatic cohorts [288, 293, 294]. On

rs-fMRI, connectivity alterations were noted in the salience, sensorimotor and default mode network [290]. Dynamic connectivity changes were also captured using chronnectomic approaches [295]. Altered glucose metabolism has been readily observed at an individual level in the presymptomatic phase using [18F]FDG PET. Frank regional hypometabolism was noted in frontotemporal, basal ganglia and thalamic and hypermetabolism was observed in peri-rolandic, superior frontal and precuneus regions [247]. MR spectroscopy in asymptomatic *C9orf72* mutation carriers captures reduced NAA/Cr, Glu/Cr and Glu/NAA ratios in the putamen and reduced Glu/NAA in thalamus in comparison to healthy non-carriers. While the majority of presymptomatic studies in ALS evaluate homogenous cohorts of GGGGCC hexanucleotide carriers, some studies have assessed admixed cohorts of *SOD1* and *C9orf72* mutation carriers, which precludes genotype-specific observations [241, 296].

2.2.3.5 Post mortem correlates

Compared to FTD, very few imaging studies in ALS offer post mortem validation and post mortem MRI is still in its infancy [297-299]. Similar to sporadic forms of ALS, *C9orf72* patients are predominantly characterised by an underlying TDP-43 pathology [300, 301]. The patterns of atrophy observed in vivo on neuroimaging mirror the distribution of TDP-43 pathology post mortem [302-304]. C9+ ALS patients suffer from substantial motor neuron loss and TDP-43 positive neuronal cytoplasmic inclusions (NCI) in the spinal anterior horns [305], additionally they have abundant p62 positive inclusions in non-motor regions, such as the prefrontal cortex, the CA4 hippocampal subfield, basal ganglia as well as granular cell layer of cerebellum [305, 306]. A significant burden of glial inclusions, predominantly in oligodendrocytes, were also observed in the prefrontal cortex, precentral gyrus and spinal cord of C9+ALS patients compared to sporadic ALS [307]. The histomorphological features of *C9orf72*-associated FTD is consistent with TDP-43 type B pathology [308, 309], but a small subset of patients also exhibits characteristics of TDP-43 type A or an overlap of type A and type B, which is thought to be relatively unique to *C9orf72* [309-311]. A presumed unique feature of C9+ ALS-FTD is the presence of cytoplasmic and intranuclear inclusions (NII) immunostaining positive for p62 which are TDP-43 negative. These have been observed in the cerebellum, hippocampus and neocortex of both ALS and FTD patients [229, 261, 306, 308, 309, 312, 313]. Similarly to imaging studies, *C9orf72*-associated thalamic degeneration is also

readily observed post mortem [314]. Also mirroring neuroimaging findings [237, 266] focal neuronal loss has been detected in the anterior cingulate, amygdala and medial pulvinar thalamus of C9+FTD patients [315]. DRP inclusions and RNA foci appear relatively diffusely across multiple brain regions and do not follow atrophy patterns. RNA foci were observed in the Purkinje cells of the cerebellum [316] and DRP inclusions were observed in the cerebellum, frontal lobes, occipital cortex, hippocampus, and more rarely in subcortical regions irrespective of the ante mortem clinical phenotype [311, 317].

2.2.3.6 Sample size consideration

The total number of symptomatic C9+ patients included in structural MRI ranges from *n*=6 [275] to *n*=64 [265]. In diffusion tensor imaging (DTI) studies, the sample size of symptomatic carriers ranges from *n*=4 [277] to *n*= 26 [245] and cohorts participating in resting-state functional MRI (rs-fMRI) studies are even smaller, ranging from *n*=7 [276] to *n*=19 [226]. PET studies using [18]-FDG, included cohorts from *n*=5 [215] to *n*= 22 [278]. The only identifiable SPECT study included 4 patients [215]. The number of presymptomatic C9+ subjects varies considerably in different studies, ranging between *n*=7 [218, 224] and *n*= 83 [262]. Currently, the largest total number of *C9orf72* patients in a single-centred study is *n*=40 [223] compared to a multicentre study including as many as 137 subjects [262]. Recruitment of large *C9orf72* cohorts is challenging due to the relatively low incidence of the mutation compounded by the difficulty that not all ALS and FTD patients are routinely tested depending on local guidelines, and sporadic patients in particular are less likely to be screened. The inclusion of mutation carriers with ALS into neuroimaging studies is additionally hampered by disease-specific factors such as sialorrhoea, dyspnoea and orthopnoea. Frank behavioural impairment, such as marked apathy or disinhibition makes recruitment into research studies also difficult. The combination of these factors may explain the relatively limited small sample size of single-centre *C9orf72* neuroimaging studies. (**Tables 2-1, 2-2 and 2-3**)

2.2.3.7 Reference groups

In the majority of studies, C9+ patients are not only contrasted to age-matched healthy individuals, but also to *C9orf72*-negative patients (C9-) in order to evaluate genotype-specific signatures [226-228, 230, 236, 246, 248, 266, 267, 278]. C9- sporadic ALS patients with neuropsychological deficits have also been used as controls to demonstrate

distinct patterns of extra-motor involvement in C9+ ALS patients [224, 226]. FTD studies often use other genetic variants such as *GRN* and *MAPT* carriers as reference groups to ascertain *C9orf72* specific imaging features [237, 259, 260, 268, 270, 271, 273, 274, 277]. In presymptomatic studies, hexanucleotide carriers are either compared to age-matched healthy individuals [231, 247, 289, 290] or to healthy *C9of72* negative relatives in the same family [219, 223, 288, 318, 319]. Presymptomatic FTD studies also included asymptomatic *GRN* or *MAPT* carriers to showcase cerebral changes specific to *C9orf72* [264, 284, 285, 287, 292, 295, 320, 321]. However, disease controls, symptomatic patients with diagnoses other than ALS or FTD are seldom included in *C9orf72* studies. (**Tables 2-1, 2-2 and 2-3**)

2.2.3.8 Recruitment strategies and inclusion bias

The effect of inclusion bias in *C9orf72* studies is seldom acknowledged. ALS patients with marked motor disability, respiratory weakness, sialorrhea or patients with severe behavioural impairment are less likely to tolerate the duration of scanning, attending a neuroimaging facility and participate in detailed cognitive assessments. As a result, there may be an underrepresentation of patients, with severe functional disability. Clinical factors therefore may impact on the severity of radiological changes identified in imaging studies. Radiological changes associated with *C9orf72* may therefore represent relatively early-stage changes when patients are still able to tolerate scanning. The functional rating scale profile of study participants is typically presented in ALS studies, but FTD studies including an ALS group often omit the reporting of functional disability. The explicit reporting of how many genotype carriers could not tolerate scanning and the presentation of their cognitive and behavioural profile seems imperative to indicate how representative the cohort undergone imaging is of the entire C9+ population. Significant differences also exist in recruitment strategies. Soon after the discovery of GGGGCC hexanucleotide repeat expansions, a series relatively small single-centre studies have been published [216, 224, 227, 230, 231, 246, 259, 260, 267, 276, 318]. The benefit of these studies is the implementation of uniform neurological, neuropsychological batteries and standardised scanning protocols. These studies are less likely to suffer from inter-rater reliability bias. Single-centre and population-based national studies were soon superseded by large multicentre initiatives which could generate larger sample sizes, but scanning protocols had to be harmonised and clinical assessments were carried out by a multitude of raters.

2.2.3.9 Demographic profiles

The disease duration profile of patients enrolled in ALS studies varies significantly ranging between 3 months [248] to 2.8 years [224]. In the identified FTD studies, mean disease duration ranges from 2.5 years [269] to 10.8 years [275]. The considerable variation in disease duration across existing studies makes the integrative interpretation of findings challenging as symptom duration is likely to impact on the severity of cerebral findings. Presymptomatic studies also vary significantly in how early prior to expected symptom onset the subjects are assessed. Mutation carriers have been evaluated from 7.2 years [286] to 21.1 years [319] prior to estimated clinical onset. The younger the presymptomatic cohort is, the earlier changes may be detected *in vivo* and the more accurate longitudinal assessments can be performed. (**Tables 2-1, 2-2 and 2-3**)

2.2.3.10 Genetic screening

The protocol used to screen for the GGGGCC hexanucleotide repeat expansions also differ across existing studies. The vast majority of studies use repeat-primed polymerase chain reaction (PCR), and only few groups have relied on a two-step approach combining repeat-primed PCR with either fluorescent fragment-length assays or southern blot to validate the PCR assays. Furthermore, the definition for pathological repeat size also differs across the published papers. Some studies considered their subjects positive for the *C9orf72* if they had more than 23 repeats while in other studies, above 30 or 40 repeats were considered pathological [206, 218, 223, 226-228, 233, 247, 248, 267, 285-287, 290, 292, 320]. While the majority of symptomatic patients typically carry over hundred repeats, intermediate repeat length expansions of 24-30 have been identified in a subset of ALS patients [322, 323]. Accordingly, some studies may have included patients with intermediate repeat expansions while other excluded these patients. It is also noteworthy that none of the identified studies have specifically evaluated the clinical and radiological profile of intermediate repeat expansion carriers. One of the commonly asked questions whether the severity of clinical manifestations increases with increasing repeat lengths. As most studies use PCR and the exact repeat length is not established, this association has not been elucidated to date.

2.2.3.11 Imaging modalities

Neuroimaging studies of *C9orf72*-associated ALS/FTD are predominantly magnetic resonance imaging (MRI) studies. (**Tables 2-1, 2-2 and 2-3**) Structural MRI and diffusion tensor imaging (DTI) were by far the most commonly used methods to characterise the grey and white matter alterations. Cortical and subcortical grey matter (GM) changes were typically evaluated by voxel-based morphometry (VBM), cortical thickness analyses, and atlas-based segmentation approaches for subcortical and cerebellar regions. Additionally, magnetic resonance spectroscopy (MRS) has been used in the presymptomatic patients [318]. White matter (WM) integrity has been evaluated in C9orf72 repeat expansion carriers by a variety of methods including white matter density measures [324, 325], cross-sectional cord area [223], tract-based spatial statistics [206, 218, 227, 230, 260, 286, 287], and probabilistic tractography [226, 228, 245, 288]. While conventional DTI is a well-established tool in detecting WM abnormalities, neurite orientation dispersion and density imaging (NODDI) is considered a particularly sensitive tool to detect early white matter alterations [288]. There are relatively few functional brain imaging studies published in *C9orf72*, these have utilised resting-state functional MRI (rs-fMRI) [226, 266, 276, 290, 295, 324-326], positron emission tomography (PET) [246-250, 278-281] and single-photon emission computer tomography (SPECT) [215]. Resting-state functional MRI (rs-fMRI) appraises functional connectivity through a variety of approaches such as seed-based intrinsic connectivity analyses, spatial independent component analysis or dynamic connectivity methods such as the "chronnectome" approach [295]. Other imaging techniques such as arterial spin labelling MRI [320] were also explored in *C9orf72*. While existing studies have included age-matched controls and typically include age and gender as covariates, the majority of studies omit adjusting for education which may be a confounder to consider [327, 328]. Furthermore, studies using multiple patient groups seldom correct for differences in symptom duration in their analyses. Only a few studies implemented a multimodal approach which overcomes the limitations of single imaging techniques. VBM is sometimes complemented by cortical thickness analyses or TBSS by probabilistic tractography, but there is a relative paucity of multimodal studies and the characteristics of some cohorts are presented in two separate publications based on the different methods utilised. Multimodal studies not only provide a more fine-grained characterisation of cerebral change, but allow the comparative assessment of the sensitivity profile of various

approaches. For example, the combined evaluation of radial diffusivity (RD) and white matter density performed better than unimodal approaches in separating presymptomatic carriers group from controls [324]. The concomitant assessment of grey and white matter indices is particularly important in early-phase patients and presymptomatic mutation carriers as white matter changes may be detected earlier than grey matter alterations as evidenced by both cerebral [286, 329] and spinal cord studies [223]. As ferroptosis has been implicated in the pathogenesis of ALS [330, 331], but not specifically studied in *C9orf72,* quantitative susceptibility mapping (QSM) may be of particular interest in this cohort to complement findings from other modalities.

2.2.3.12 Data interpretation and statistical models

Thirteen *C9orf72* longitudinal imaging studies were identified; 12 cerebral [220] and one spinal cord study [223]. While some studies had as many as 5 follow-up assessments [225, 245, 262], the vast majority of longitudinal studies only relied on 2 time points, rendering the assessment of linear versus non-linear disease progression and the evaluation of ceiling and flooring effects impossible. Inter-scan intervals in ALS studies are typically less than 6 months [225, 245], which stands in contrast to FTD studies where follow-up interval ranges from 6 – 27 months [260, 268, 277]. A variety of statistic models were utilised to interpret radiological changes over time. Some studies calculated rate of change, expressed as the percentage loss from baseline volume, adjusted to an annualised rate, assuming linearity [261, 277]. 'Within-patient' changes were visualised using fluid registration with the non-linear warping of patients' follow-up scan to their baseline scan to generate a deformation field for each subject [260]. Another approach evaluated longitudinal changes by directly subtracting follow-up images from baseline images followed by longitudinal analysis of variance (ANOVA) to compare study groups [287]. Linear mixed effect models were the most commonly utilised models [225, 229, 245, 262, 268, 279, 321], as they accommodate irregular follow-up intervals, and is particularly suitable for studies with high attrition rates and those with non-linear trajectories of decline. Relevant covariates such as age and gender, disease duration can be readily incorporated in mixed effect models, while random between-subject effects can be accounted for using random slopes and random intercepts [268]. Other models commonly used in *C9orf72* studies include repeated measures ANOVA [224, 265],

random coefficient model with intercept [218] as well as mixed model with two levels of hierarchy [223].

2.2.3.13 Clinical profiles

Given the heterogeneity of clinical manifestations in *C9orf72* GGGGCC repeat expansions the comprehensive clinical characterisation of study participants is paramount for the development of meaningful imaging markers. Neuroimaging studies invariably include clinical assessments, but the focus of assessments varies considerably among the identified studies. (**Tables 2-1, 2-2 and 2-3**) ALS research groups often focus on motor disability [162, 332], but additional neuropsychological testing is also often performed [333, 334]. In contrast, FTD groups predominantly focus on cognition and behaviour, and motor disability is typically assessed in less detail. Clinical assessments and neuroimaging are the mainstay of *C9orf72* studies, and complementary biofluid panels, respiratory assessments, and post mortem data are seldom available [330, 331]. Patients carrying the *C9orf72* mutation have been associated with younger age of onset [335] and shorter survival if they have ALS [214, 265, 279, 336, 337]. Age of symptom onset was reported to range from 33 to 72 years old and survival ranged from 1 to 17 years [215]. Interestingly, C9+ FTD-MND patients are thought to exhibit longer survival compared to C9- FTD-MND [216]. C9+ FTD patients were also found to have a younger age of onset but comparable mean survival compared to other genetic FTD variants [336]. Intriguingly, a subset of C9+ FTD patients has been consistently reported to exhibit an exceptionally slow rates of progression [338-340], with up to 20-30 years of symptom while maintaining independence in activities of daily living [339, 341]. Psychiatric manifestations preceding FTD onset have also been consistently reported [342]. There is a consensus in the literature that in the families of C9+ patients there is a higher prevalence of ALS, FTD and psychiatric disorders [267]. Clinically, C9+ ALS patients were more likely to exhibit cognitive and/or behavioural deficits or frank FTD compared to C9-ALS patients [214, 236]. Executive function and behaviour are the most frequently evaluated neuropsychological domains in ALS, but deficits in social cognition, language, and memory are increasingly recognised [343-345]. FTD patients with *C9orf72* mutation are more likely to suffer from comorbid ALS compared to other genetic variants of FTD [336]. While C9+bvFTD have similar clinical manifestations to C9-bvFTD [346], C9+bvFTD were found to exhibit higher rates of psychotic symptoms such as

hallucinations (auditory, tactile and visual), delusions (persecutory, somatic, jealous and grandiose type) and irrational thinking compared to symptomatic non-carriers [216, 263, 347, 348]. Psychotic symptoms have been linked to cortical and subcortical degeneration in frontal, temporal, thalamic, striatal and cerebellar regions [263]. Across the genetic spectrum of FTD, genotype-specific clinico-radiological correlations have been proposed; delusions were linked to left frontal cortical atrophy, and anxiety associated to cerebellar atrophy in C9+ while anxiety in GRN carriers were mapped to GM atrophy in posterior default mode network (DMN) structures [349]. Not only are psychotic symptoms strongly associated with the clinical phenotype of *C9orf72* but it was also found that first- and second degree relatives of C9+ patients are at a higher risk of developing schizophrenia and mental health disorders including autism spectrum disorder [350]. C9+ FTD patients were also found to display eating disorders [216, 347]. In contrast to non-carriers, C9+ patients are also more likely to exhibit visuospatial dysfunction [335], working memory impairments [216] and impaired episodic memory which have been linked to frontotemporal and posterior cingulate degeneration [351].

2.2.4 Discussion

Hexanucleotide expansion carriers younger than 40, already exhibit considerable cortical and subcortical changes, decades before projected symptom onset [219, 220, 262, 288-290]. Non-specific neurodegenerative changes, such as ventricular volume expansion are also readily captured many years prior to symptom onset [321]. Arterial spin labelling detects reduced cerebral blood flow in insular, orbitofrontal, cingulate, inferior parietal and middle temporal regions, as early as 12.5 years prior to symptom onset [320]. Thalamic shape deformations can be noted 5 years prior to symptom onset primarily affecting the anterior aspect of the structure [352]. The observations from young cohorts of asymptomatic mutation carriers are sometimes interpreted as a neurodevelopmental phenomenon as opposed to a neurodegenerative process [220, 222, 353]. This notion is also supported by FTD studies, where presymptomatic changes are invariably observed much earlier in *C9orf72* mutation carriers than in GRN or MAPT carriers [285]. The earliest grey matter changes are typically detected in the insula, thalamus and posterior cortical regions, as early as 25 years before expected symptom onset, followed by frontal and temporal lobe atrophy 20 years before projected symptom onset and 10 years prior to estimated symptom onset cerebellar changes have been detected [285]. White matter

changes are also detected much earlier in *C9orf72* carriers than in *GRN* and *MAPT* mutation carriers; these may be observed as early as 30 years before symptom onset, preferentially affecting posterior cerebral tracts such as the posterior thalamic radiation, the splenium of corpus callosum or the posterior corona radiata [292]. Longitudinal presymptomatic *C9orf72* studies did not capture progressive changes over a 2-year interval [287]. Taken together, the presymptomatic literature of *C9orf72* suggests a slowly progressive process which is already present at a young age, several decades before symptom manifestation [341, 354]. Some studies suggest that presymptomatic C9 positive patients exhibit relatively similar progression rates to C9 negative patients [289, 290] while others [262] identified faster cortical thinning and surface area loss. One of the deliverables of longitudinal imaging in ALS is to verify proposed propagation theories such as trans-synaptic spread, stage-wise propagation, network-wise spread etc. [304, 355-360]. Biological propagation in *C9orf72* repeat expansion carriers have been specifically investigated by longitudinal studies with regards to cell-to-cell dipeptide repeat proteins (DPRs) transmission [361, 362]. While animal studies have invariably shown focal DPR toxicity, human post mortem studies have been less successful in demonstrating a clear association between regional DPR and anatomical vulnerability [363]. The concentration of soluble DPRs is thought to be lower in clinically affected anatomical regions such as the frontal and temporal cortices and the largest DPR load can be identified in the cerebellum [364]. While there is a suggestion that soluble and insoluble DPR levels define anatomical vulnerability in *C9orf72* this has not been demonstrated by imaging studies to date. Reports on cerebellar atrophy in *C9orf72* are inconsistent, some linked to *C9orf72* status to cerebellar degeneration [226, 228] while others found no evidence of preferential cerebellar degeneration in hexanucleotide repeat carriers [224, 269, 365]. Cerebellar changes in ALS has been consistently identified by post mortem studies[366], but the imaging literature is less consistent. Some functional MRI studies suggested a compensatory role in response to motor cortex degeneration, but other did not confirm this observation [367-369].

While disease burden based have been used to predict survival in symptomatic cohorts [370-372], similar algorithms have not been applied to presymptomatic imaging data to predict age of symptom manifestation. Cerebral studies [219] suggested that patterns of white matter pathology may be predictive of the future phenotype. In spinal cord studies [223] CST FA reductions were exclusively found in presymptomatic C9+ carriers related

to symptomatic ALS patients and not in carriers related to FTD patients. Offering genetic testing to symptomatic patients with ALS/FTD and their relatives at risk of harbouring the *C9orf72* repeat expansions has a number of important practical ramifications [221, 373]. Beyond the need of formal genetic counselling, the emergence of antisense oligonucleotide therapies necessitates discussions around clinical trial participation. At some ALS centres, genetic testing is only offered to ALS patients thought to have 'familial ALS'. The lack of consensus on what defines a positive family history makes the delineation between familial and sporadic cases difficult [374-376]. As 4-7% of seemingly sporadic cases also carry *C9orf72* mutation [209, 210], there is a compelling argument that genetic testing and counselling should be offered to all symptomatic ALS patients. Screening for common ALS and FTD causing mutations in symptomatic patients will help patient recruitment into future ASO trials. The screening of asymptomatic, at-risk populations for *C9orf72* raises a number of ethical considerations and requires robust genetic counselling framework. The meticulous mapping of presymptomatic changes however will help to define the optimal timing of ASO interventions. By the time ALS patients fulfil current diagnostic criteria relatively widespread neurodegenerative changes have occurred [377] and accordingly, at risk gene carriers may benefit from earlier recruitment into ASO trials in the future. There is ample evidence that, despite the current lack of effective disease-modifying therapies, many newly diagnosed patients would seek genetic testing if offered [373, 376]. Similarly, presymptomatic genetic testing is well received by at-risk individuals if it is delivered by a dedicated team of experienced genetic counsellor, geneticist, neurologist and research team [378]. The availability of adequate pre-test and post-test counselling, long-term follow-up for those who elect to learn of their results and follow-up for those who tested negative are important considerations before providing genetic testing [221, 378].

The key shortcoming of existing C*9orf72* neuroimaging studies include sample size limitations, the paucity of longitudinal studies, the rarity of studies following presymptomatic subjects beyond symptom manifestation, the inclusion of control groups without genetic testing, the scarcity of spinal cord imaging studies, the absence of *C9orf72*-associated PLS studies, and the lack of studies interpreting single subject imaging data [58, 60, 379-383]. The limitations of existing studies may lead to misconceptions such as the exclusive association of frontotemporal changes in ALS with *C9orf72,* despite evidence of widespread extra-motor change in C9 negative cohorts [205,

206, 228, 258]. Future *C9orf72* imaging studies should aspire to recruit young asymptomatic GGGGCC hexanucleotide carriers to evaluate progression patterns longitudinally, develop and validate personalised prognostic models, assess compensatory changes and investigate if patterns of presymptomatic radiological changes can foretell the future phenotype [220, 228, 367].

2.2.5 Conclusions

GGGGCC repeat expansion carriers in *C9orf72* are a unique cohort with distinctive clinical, imaging and pathological features. While imaging studies have already contributed important academic insights, future studies need to recruit larger number of participants, follow asymptomatic carriers from a young age to beyond symptom manifestation, perform both motor and neuropsychological assessments, support radiological observations with biofluid analyses, adopt multi-timepoint longitudinal study designs and implement multimodal imaging protocols in order to develop viable biomarkers with practical clinical utilities.

Author(s) and Year of publication	Study design / Imaging technique	Sample	Mean time to symptom onset (years)	Assessment battery	Main conclusion
Lee et al. (2017) [290]	Cross-sectional, retrospective, single-centre/ Structural: VBM; DTI: TBSS; rs-fMRI: seed correlation analyses	VBM: 15 pC9+, 67 HC DTI: 12 pC9+, 30 HC rs-fMRI: 13 pC9+, 30 HC	8.2	MMSE, FTLD-CDR, NPI, IRI, GDS, CVLT, Benson figure 10 min recall, Benson figure copy, visual object and space perception battery, calculations, abbreviated BNT, wide range achievement test 4, digit span forward and backward, modified trails, Stroop colour-naming test, letter fluency, semantic fluency, design fluency, comprehensive affective testing system, face and affect matching	- pC9+ showed GM volume deficits in similar areas as symptomatic C9+bvFTD with major foci in cingulate, insula, thalamus, and striatum. - Reduced white matter integrity in the corpus callosum, cingulum bundles, corticospinal tracts, uncinate fasciculi and inferior longitudinal fasciculi. - Intrinsic connectivity deficits were detected in all four networks, worse in salience and medial pulvinar thalamus-seeded networks
Querin et al. (2019)[384]	Longitudinal, prospective, single-centre/ Structural: CSA; DTI: FA, RD, AD, MD	40 pC9+, 32 pC9-	17.95	ALSFRS-R, MMSE, MDRS, FAB	- At baseline, pC9+ >40 yo displayed WM atrophy of C2 to C7 and no GM or diffusivity changes. - At 18 month follow-up, WM atrophy was accompanied by progressive CST FA reduction in pC9+ >40 yo. - Only pC9+ > 40 years with positive family history of ALS had marked CST FA reduction at baseline.
Papma et al. (2017)[286]	Cross-sectional,	18 pC9+, 15 pC9-	7.2	MMSE, FAB, BDI, NPI, CBI-R, 60-item BNT, Semantic	- Subtle decline in language, attention and executive function compared to HC

	prospective, single-centre/ Structural: VBM; DTI: TBSS			Association Test verbal subtask, categorical (animal) and letter fluency, TMT A and B, Stroop colour-word test I and II, Stoop III, letter digit substitution test, Dutch version of Rey Auditory Verbal learning test learning and recall, WAIS-III digit span total, WAIS-III block design, Royal clock drawing, Happé cartoon task, Ekman 40 faces test	- WM alterations in tracts connecting frontal lobe (ILF, uncinate fasciculus), thalamic radiation, tracts associated with motor functioning (CST, corona radiata, internal and external capsule) - GM atrophy in subset of C9+ >40yo in thalamus, cerebellum and parietal and temporal regions.
Panman et al. (2019)[287]	Longitudinal, retrospective, single-centre/ Structural: VBM, CTh analyses; DTI: TBSS	12 pC9+, 15 pMAPT+, 33 pGRN+, 53 nc	Not available	FTD-CDR, MMSE, NPI-Q, MMSE	- Lower GM volume in cerebellum, thalamus, insula, frontal and temporal regions; cortical thinning in bilateral precentral gyrus and right superior parietal lobule - Lower FA and higher MD in frontotemporal tracts (bilateral CST and anterior thalamic radiation, right inferior fronto-occipital fasciculus and SLF) and entire skeleton respectively - No longitudinal changes in GM or WM metrics.
Popuri et al. (2018)[284]	Cross-sectional, prospective, single-centre/ Structural:	15 pC9+, 9p GRN+, 23 pC9-, 15pGRN-	13.7	MMSE, FAB, FBI	- *C9orf72:* cortical thinning in left frontal, left parietal, left occipital, left cingulate and right temporal and right parietal lobes. Atrophy of bilateral thalamus and

	CTh analyses, subcortical volumetry				left caudate but no cerebellar atrophy detected - *GRN*: No cortical thinning or atrophy of cerebellar or subcortical structures in presymptomatic stage
Caverzasi et al. (2019)[289]	Cross-sectional, prospective, single-centre/ Structural: CTh analyses, local gyrification index	19 pC9+, 67 HC	8.2	n/a	- Lower local gyrification index in left subgenual anterior cingulate cortex, left precentral gyrus, right inferior parietal lobule and right superior occipital gyrus - No concomitant cortical thickness abnormality - No correlation between CT and lGI suggesting gyrification captures a feature distinct from cortical thickness
De Vocht et al. (2020)[247]	Cross-sectional, prospective, single-centre/ Structural MRI: VBM; PET: glucose metabolism	17 pC9+, 25 HC	Not available	MMSE, BDI, ECAS	- Hypometabolism in frontotemporal regions, basal ganglia, and thalami - Relative hypermetabolism in the peri-Rolandic region, superior frontal gyrus, and precuneus cortex.
Westeneng et al. (2017)[318]	Cross-sectional, prospective, single-centre / Proton	11 pC9+, 18nc	Not available	n/a	- Lower tNAA/Cr, Glu/tCr and Glu/tNAA ratios in left putamen, lower Glu/tNAA in left thalamus

	MRSI: Metabolite ratios				
Montembeault et al. (2020)[319]	Cross-sectional, retrospective, multi-centre/ Structural: VBM	38 pC9+, 22pC9-	21.1	MMSE, FAB, Hayling Sentence Completion test part A and B	- pC9+ > 40 yo displayed higher error scores and longer time to completion of task - GM volume in left cerebellar lobule VI, right cerebellar lobule VIIIb, IX, VI correlated with HSCT time to completion. - pC9+ patients: Peak significance observed in left vermis crus II and right vermis VIIb.
Wen et al. (2019)[288]	Cross-sectional, prospective, multi-centre/ Structural: atlas-based volumetry; DTI: atlas-template tractography; NODDI: NDI, ODI and FWF maps	38 pC9+, 29 pC9-	18.6	MMSE, MDRS, FAB	- pC9+: WM abnormalities in 10 tracts with NODDI compared to only 5 with DTI metrics and free water faction increased in 13 regions on NODDI compared to only 11 regions of atrophy detected on structural MRI

Bertrand et al. (2018)[219]	Cross-sectional, prospective, multi-centre / Structural: ROI volumetry; DTI: FA, MD, RD, AD	41 pC9+, 39 pC9-	19.3	MMSE, MDRS, FBI, FAB, finger dexterity, melokinetic apraxia, nonrepresentational gestures, intransitive gestures, Mini-SEA, Benson figure, free and cued recall test, BNT, category and letter fluency test	- pC9+ <40yo had lower praxis scores and intransitive gesture scores, atrophy in 4 cortical regions of interest and in the right thalamus, and white matter alterations in 2 tracts.
Mutsaerts et al. (2019)[320]	Cross-sectional, prospective, multi-centre/ ASL MRI: VBM	34 pC9+, 55pGRN+, 18pMAPT+, 113nc	11	MMSE, CBI-R, FDRS	- Reduced CBF in bilateral insulae/orbitofrontal cortices, anterior cingulate/paracingulate gyri, and inferior parietal cortices, left middle temporal gyrus, driven by C9+ subgroup, seen as early as 12.5 years prior to expected symptom onset - Lower CBF in presymptomatics closer to and beyond their expected age of symptom onset
Premi et al. (2019)[295]	Cross-sectional, prospective, multi-centre/ rs-fMRI: "chronnectome" approach	45pMAPT+, 122 pGRN+, 82 pC9+, 223 nc	13.8	CBI-R, MMSE, TMT A and B	- Diminished dynamic fluidity, visiting less meta-states, shifting less often across them, and travelling through a narrowed meta-state distance
Premi et al. (2017)[385]	Cross-sectional,	108 presympto	Not available	FDRS, CBI-R, MMSE, logical memory subtest of Weschler	- Greatest GM atrophy in *C9orf72* patients

	retrospective, multi-centre/ Structural: composite measure of GM volume	matic, 123 nc		memory scale revised with immediate and delayed recall, digit span forward and backward from Weschler memory scale-revised, digit symbol task, TMT A and B, short version of BNT, category (animal) fluency, letter fluency, WASI block design task	- TMEM106B enhances the benefit of cognitive reserve on brain structure
Feis et al. (2019)[325]	Cross-sectional - classification study, retrospective, multi-centre/ Structural: GM and WM density; DTI: TBSS; rs-fMRI: functional connectivity features	8 pMAPT+, 35 pGRN+, 12 pC9+, 48 nc	Not available	n/a	- Multimodal carrier-control model combining RD and WM density features was best performing model (AUC =0.684) to separate FTD mutation from controls
Feis et al. (2019)[324]	Longitudinal - classification study, prospective, multi-centre/	8 pMAPT+, 35 pGRN+, 12 pC9+, 48 nc	Not available	MMSE	- Converters had stronger classification score increase over time than non-converters. - MRI-based classification scores remains similar to controls until they are close to symptom onset

	Structural: GM and WM density; DTI: TBSS; rs-fMRI: functional connectivity features				
Lulé et al. (2020) [386]	Longitudinal, prospective, single-centre/ DTI: WBSS (FA maps)	22pC9+, 15pSOD1 +, 91 HC	14.2	HADS, ACSA, ECAS, RWT phonetic fluency, design fluency, Doors test, PAL, TMT B	- pC9+ showed reduced verbal and non-verbal memory performance - Lower FA in inferior frontal and orbitofrontal cortical areas - No longitudinal changes in cognitive assessments or neuroimaging over a 12-month period

Table 2- 1: Presymptomatic neuroimaging studies in *C9orf72*

Author(s) and year of publication	Study design/ Imaging technique	Sample	Assessment battery	Main conclusion
Rohrer et al. (2015)[285]	Cross-sectional, retrospective, multi-centre/ Structural: Cortical and subcortical volumetry	40 symptomatic FTD (16 C9+, 11 MAPT, 13 GRN), 78 presymptomatic carriers (18 pC9+, 15 pMAPT+, 45 pGRN+), 102 presymptomatic nc (24 pC9-, 18 pMAPT-, 60 pGRN-)	FDRS, CBI-R, MMSE, logical memory subtest of Weschler memory scale revised with immediate and delayed recall, digit span forward and backward from Weschler memory scale-revised, digit symbol task, TMT A and B, short version of BNT, category (animal) fluency, letter fluency, Wechsler Abbreviated Scale of Intelligence block design task	- Sequential pattern of atrophy in thalamus, insula, posterior cortical areas (25 years prior), frontal and temporal lobes (20 years prior) and cerebellum (10 years prior to symptom onset)
Rittman et al. (2019) [326]	Cross-sectional, retrospective, multi-centre/ rs-fMRI: connection strength, efficiency and connectedness of network connectivity	24 symptomatic FTD (11 C9+, 11MAPT, 6GRN), 68 presymptomatic FTD (19 C9+, 13 MAPT, 38 GRN), 80nc	MMSE, immediate memory, delayed memory, forward and backward digit span, TMT B and A, digit symbol task, Boston naming task, category and letter fluency, block design task	- Decreased connectivity in frontal lobes, temporal lobes, occipital lobes and cingulate cortices, cerebellum, insula cortices - Reduced efficiency in occipital cortex
Jiskoot et al. (2018) [292]	Cross-sectional, retrospective, multi-centre/	54 C9orf72, 30 MAPT, 56 GRN, 115 nc	CBI-R, MMSE	- Earliest changes in the posterior thalamic radiation, splenium of the corpus callosum and posterior corona radiata

	DTI: FA, MD, RD, AD			- Later presymptomatic stage changes in internal capsule
Fumagalli et al. (2018) [387]	Cross-sectional, retrospective, multi-centre/ Structural: VBM, visual rating scales	63 symptomatic, 132 presymptomatic, 143 nc	FDRS, CBI-R, MMSE, logical memory subtest of Weschler memory scale revised with immediate and delayed recall, digit span forward and backward from Weschler memory scale-revised, digit symbol task, TMT A and B, short version of BNT, category (animal) fluency, letter fluency, WASI block design task	- Widespread pattern of atrophy in *C*9+ - No atrophy detected using visual rating scales
Tavares et al. (2019) [321]	Longitudinal, retrospective, multi-centre/ Structural: ventricular volume	18 symptomatic, 46 presymptomatic (13 pC9+, 29 pGRN+, 4 pMAPT+), 56 nc	FDRS, CBI-R, MMSE, logical memory subtest of Weschler memory scale revised with immediate and delayed recall, digit span forward and backward from Weschler memory scale-revised, digit symbol task, TMT A and B, short version of BNT, category (animal) fluency, letter fluency, WASI block design task	- Greater annualised rates of ventricular volume expansion in presymptomatic carriers
Cury et al. (2019) [352]	Cross-sectional, retrospective, multi-centre/ Structural: LDDMM	37 symptomatic carriers 76 presymptomatic carriers, 89 nc	n/a	- Shape deformation in anterior region of thalamus (>5 years before symptom onset)

Walhout et al. (2015) [231]	Cross-sectional, prospective, single-centre/ Structural: CTh analyses, volumetry; DTI: WM connectivity	19 pC9+, 23 pC9-, 14 C9+ALS, 28 HC	ALSFRS-R, ECAS, RAB, VFI	- C9+ALS displayed cortical thinning in primary motor regions, thalami, putamen, right caudate, left pallidum, nuclei accumbens, hippocampi, amygdala, ventricular and WM integrity loss in CST and CC. - pC9+: cortical thinning in temporal, parietal, occipital regions, left caudate and putamen atrophy and sparing of primary motor cortex atrophy and loss of WM connectivity globally, CST, CC
Floeter et al. (2018) [218]	Longitudinal, prospective, single-centre/ DTI: TBSS, fibre tracking	28 C9+, 28 HC	ALSFRS-R, letter fluency, FBI	- pC9+: no difference - C9+: Decreased FA in rostral, mid-portions of CC, cingulum, superior longitudinal fasciculus, uncinate fasciculus, anterior limb of anterior capsule and CST. FA reductions extended into subcortical WM and temporal lobes longitudinally. - C9+ALS: lower FA in CST, premotor segment of CC and increased MD in CST at baseline and follow-up - C9+bvFTD and ALS-FTD: reduced FA and increased MD in uncinate fasciculus

Sellami et al. (2018) [349]	Cross-sectional, retrospective, multi-centre/ Structural: VBM	45 symptomatic (12 GRN, 23 C9of72, 10 MAPT), 122 presymptomatic (63 pGRN+, 37 pC9+, 22 pMAPT+)	FRS, neuropsychological symptoms by structured interview, NPS scale	- Delusions in C9+ carriers were associated with left frontal cortical atrophy - Anxiety in C9+ carriers correlated with cerebellar atrophy
Olney et al. (2020) [291]	Cross-sectional, retrospective, multi-centre/ Structural: Voxel-wise maps volumetry, atlas-based brain parcellation	102 presymptomatic nc, 103 presymptomatic carriers, 43 mildly/questionably symptomatic carriers, 72 symptomatic carriers	CDR plus NACC FTLD, MoCA, NPI-Q, GDS, BIS, RSMS, UPDRS, PSPRS, Craft story immediate and delayed recall, 10 minute recall for Benson complex figure, copy of Benson figure, CVLT, MINT, letter ("L" and "F") and category (animals and vegetables) fluency, forward and backward digit span, trail making A and B	- pC9+: reduced thalamic volume on right and left peri-insular region. - Mildly symptomatic C9+: reduced thalamic volume and in patchy regions in frontal and temporal lobes. - Symptomatic C9+: fairly diffuse regions of overlap including thalamus, bilateral insula and medial parietal regions
Le Blanc et al. (2020) [262]	Longitudinal, retrospective, multi-centre/ Structural: vertex-wide CTh and CSA	54 C9+, 83 pC9+, 249 nc	MMSE, CBI	- Symptomatic C9+: symmetrical widespread cortical thinning with sparing of primary sensory cortices and occipital lobes. Lower CSA in temporal lobes - pC9+: decreased cortical thickness and CSA in medial frontal and parietal regions - Faster cortical thinning in C9+ carriers starting in early 30s and steady progression.

Cash et al. (2018) [264]	Cross-sectional, retrospective, multi-centre/ Structural: VBM	47 symptomatic (25 C9orf72, 12 GRN, 10 MAPT), 128 presymptomatic (40 pC9+, 65 pGRN+, 23 pMAPT+), 144 nc	MMSE	- C9+: widespread diffuse cortical involvement, subcortical structures involving thalamus, hippocampus, amygdala, basal ganglia and superior posterior area of cerebellum - pC9+: same cortical regions but lesser extent, bilaterally in thalamus, right superior posterior cerebellum (crus I), superior temporal and inferior frontal regions
Sudre et al. (2017) [388]	Cross-sectional, retrospective, multi-centre/ Structural: WM hyperintensities - automatic detection and quantification algorithm	43 symptomatic, 61 presymptomatic, 76 nc	n/a	- No WMH found in symptomatic or presymptomatic C9+
Gorges et al. (2017) [241]	Cross-sectional, prospective, single-centre/ Structural: volumetry; DTI: DTI-based fibre tracking	251 sporadic ALS, 19 symptomatic ALS mutation carriers, 32 presymptomatic ALS mutation carriers, 112 HC	ALSFRS-R	- Anterior and posterior hypothalamic atrophy seen both sporadic and mutation carriers ALS

Table 2- 2 Combined symptomatic and presymptomatic studies in *C9orf72*.

Author(s) and year of publication	Study design/ Imaging technique	Sample	Mean disease duration (years)	Assessment battery	Main conclusion
Byrne et al. (2012) [214]	Cross-sectional, retrospective, single-centre/ Structural: VBM	21C9+, 170 C9-	1.2	ALSFRS-R, FrSBe, Stroop colour-word teat, Brixton Spatial anticipation test, backward digit span, category fluency, phonemic verbal fluency, local memory, verbal paired associate, auditory delayed recognition task, California Verbal learning tests, Rey-Osterrieth Complex figure	- Lower age at disease onset, shorter survival and more co-morbid FTD - Distinct pattern of non-motor cortex involvement in right inferior frontal gyrus, right superior frontal gyrus, left anterior cingulate gyrus, right precentral gyrus
Lee et al. (2014) [266]	Cross-sectional, retrospective, multi-centre/ Structural: VBM; rs-fMRI: seed-based intrinsic connectivity analyses	14 C9+ (9bvFTD, 5 FTD-MND), 14 C9- (9bvFTD, 5 FTD-MND), 14 HC	7.8	FTLD-CDR, NPI	- C9+bvFTD had greater atrophy in bilateral medial pulvinar thalamic nuclei, post central gyrus, precuneus and lateral parietal cortex - C9+: decreased connectivity in the salience and sensorimotor networks. - C9+: Correlation between decreased salience network connectivity and atrophy in the left medial pulvinar thalamic nucleus
Boeve et al. (2012) [215]	Cross-sectional, retrospective, multi-centre/	43 C9+, 16 MAPT	Not available	MMSE, DRS-2, ALS-Cognitive Behavioural screen, digit span, TMT, Stroop colour-word test, BNT,	- Bilateral frontal abnormalities with variable degrees of parietal with or without temporal changes

	Structural: z-score maps or STAND-maps; FDG-PET: hypometabolism patterns; SPECT: hypoperfusion patterns	+, 10 GRN, 290 sporadic FTD/ALS		category fluency, delayed recall from Logical memory, visual reproduction, auditory verbal learning test, CVLT-II, block design, judgement of line orientation, reading subtest from Wide range achievement test, 3rd edition	
Agosta et al. (2017) [226]	Cross-sectional, prospective, multi-centre/ Structural: CTh analyses, subcortical volumes; DTI: TBSS, tractography; rs-fMRI: independent components for functional connectivity	19 C9+MND, 67 C9-MND, 22 HC	1.4	MMSE, ALSFRS-R, caregiver report, FBI, ALS-FTD questionnaire, Hamilton Depression rating scale, Raven coloured progressive matrices, written and spoken phonemic and semantic fluency tests, fluency indices, digit span backward, cognitive estimation task, Wisconsin card sorting test, Weigl's sorting test, digit span forward, RAVLT immediate and delayed recall, oral noun confrontation naming subtest of BADA	- C9+ vs sporadic: cerebellar and thalamic atrophy, greater involvement of inferior fronto-occipital fasciculus bilaterally - C9+ vs early-sporadic: greater cortical thinning in right lateral occipital cortex and cuneus; enhanced visual network functional connectivity - C9+ vs sporadic-motor: greater damage of entire CC - C9+ vs sporadic-cognitive: greater damage to right SLF and uncinate; decreased functional connectivity in sensorimotor network
Westeneng et al. (2016) [228]	Cross-sectional, prospective, single-centre/ Structural: CTh analyses, subcortical volumes;	156 C9-ALS, 14 C9+ ALS, 92 HC	1	ALSFRS-R, VFI, FAB	- Cortical thinning in precentral gyrus, bilateral pars opercularis, fusiform, lingual, isthmus-cingulate and superior parietal cortex and lower volumes of right hippocampus and bilateral thalamus

	DTI: probabilistic tractography (FA and RD)				- Reduced WM integrity of inferior and superior longitudinal fasciculi compared to C9- - Small proportion of C9- had 'C9+' neuroimaging phenotype and lower performance on the frontal assessment battery
Omer et al. (2017) [206]	Cross-sectional, prospective, single-centre/ Structural: GM morphometry analyses, ROI GM analyses; DTI: TBSS	20 FTD, 10 C9+ALS-FTD, 10C9-ALS-FTD, 20 ALS, 40 HC	Not available	ALSFRS-R, FrSBe, Stroop colour-word teat, Brixton Spatial anticipation test, backward digit span, category fluency, phonemic verbal fluency, local memory, verbal paired associate, auditory delayed recognition task, California Verbal learning tests, Rey-Osterrieth Complex figure	- ALS-FTD show widespread and symmetrical patterns of atrophy - C9- ALS-FTD had more extensive GM and WM pathology than C9+ patients - C9+ALS-FTD demonstrated orbitofrontal, cingulate and opercular WM pathology
Mahoney et al. (2015) [277]	Longitudinal, retrospective, single-centre/ Structural: BBSI; DTI: FA, MD, AD, RD maps atlas-based and mask-based analysis	4 C9+bvFTD, 8MAPT+bvFTD, 11 sporadic bvFTD, 18 HC	9.3	MMSE, WASI, VIQ, PIQ, recognition memory test, words, faces, graded naming test, graded arithmetic test, visual object space perception, DKRFS colour naming, DKEFS ink colour naming, TASIT, emotion recognition, social inference task	- At baseline, C9+ displayed lower FA in right and left superior cerebellar peduncle compared to controls - Longitudinally, C9+ showed largest FA reduction in right paracallosal cingulum bundle and increase in MD of left uncinate fasciculus.

Bede et al. (2013) [227]	Cross-sectional, prospective, single-centre/ Structural: CTh analyses, VBM; DTI: TBSS	30 C9-ALS, 9 C9+ ALS, 44 HC	2.3	ALSFRS-R, FrSBe, Stroop colour-word teat, Brixton Spatial anticipation test, backward digit span, category fluency, phonemic verbal fluency, local memory, verbal paired associate, auditory delayed recognition task, California Verbal learning tests, Rey-Osterrieth Complex figure	- C9+ have overlapping WM pathology with C9- ALS in body of CC, superior corticospinal pathway, but further WM pathology in genu of CC, anterior commissure and bilateral thalami - C9+ showed symmetrical cortical atrophy of bilateral dorsolateral prefrontal cortices, insular cortices, fusiform gyri, supramarginal cortices, lateral occipital cortices, precuneus, temporal poles, pars triangularis, pars opercularis of inferior frontal gyri - C9+ ALS displayed extensive orbitofrontal, opercular and temporal changes on VBM - C9- ALS was confined to CST and cerebellar pathways with limited extra-motor expansion
Bede et al. (2013) [230]	Cross-sectional, prospective, single-centre/ Structural: Volumetric, vertex-wise approaches; DTI: TBSS	9 C9+ALS, 30 C9-ALS, 44 HC	2.3	ALSFRS-R, FrSBe, Stroop colour-word teat, Brixton Spatial anticipation test, backward digit span, category fluency, phonemic verbal fluency, local memory, verbal paired associate, auditory delayed recognition task, California Verbal learning tests, Rey-Osterrieth Complex figure	- C9+ALS displayed more extensive basal ganglia pathology including volume loss of bilateral thalami, left caudate, left putamen, bilateral hippocampi - C9-ALS displayed volume loss in left caudate, left hippocampus and right accumbens - C9+ and C9- showed vertex-wise differences in superior and inferolateral aspect of right thalamus

					- C9+ ALS showed reduced thalamic FA and increased AD, MD and RD of thalamus and hippocampus - C9- showed lower FA in globus pallidus and increased AD, RD and MD in accumbens nuclei.
Floeter et al. (2016) [224]	Longitudinal, prospective, single-centre/ Structural: Volumetric, CTh analyses	27 C9+, 22sALS, 28 HC	2.8	DRS-2, FBI, FrSBe, ALSFRS-R, D-KEFS fluency subtest	- pC9+: no difference - C9+: greater ventricular expansion, thalamic atrophy and diffuse patchy cortical thinning - C9+ ALS vs sALS: more thinning in superior frontal gyrus and lateral orbitofrontal cortex - No longitudinal cortical thickness changes; progressive ventricular expansion over 6 months follow-up
Ly et al. (2019) [250]	Cross-sectional, prospective, single-centre/ Structural MRI/PET: regional SUVRs	9 C9+ALS, 18 HC	2.5	ALSFRS-R, MMSE, ALS-CBS, trail making A and B, letter-number sequencing, category fluency, logical memory subtest from Wechsler Memory Scale III	- Increased AV-1451 binding in entorhinal cortex
Bevan-Jones et al. (2018) [280]	Cross-sectional, prospective, single-centre/ Structural MRI/PET: brain atlas-based	1 C9+ bvFTD, 13 HC	3	ACE-R, MMSE, CBI-R	- Increased ligand binding in left fusiform gyrus, left medial anterior temporal lobe, left middle and inferior temporal gyri, left lateral inferior temporal lobe - Widespread GM loss in temporal lobes.

	parcellation technique				
Whitwell et al. (2012) [259]	Cross-sectional, retrospective, single-centre/ Structural: VBM, ROI atlas-based parcellation	19 C9+ bvFTD, 25 MAPT, 12 GRN, 20 sporadic FTD, 40 HC	2.7	n/a	- C9+: symmetric atrophy involving dorsolateral, medial and orbitofrontal lobes, with additional loss in anterior temporal lobes, parietal lobes, occipital lobes and cerebellum
Whitwell et al. (2015) [268]	Longitudinal, retrospective, single-centre/ Structural: BBSI, TBM-SyN	21 MAPT, 11 GRN, 11 C9+, 15 sporadic FTD	4	CDR-SB, MMSE	- C9+: frontal and temporal lobe atrophy; greater rates of atrophy in left cerebellum and right occipital lobe than MAPT - Sporadic FTD: greater rates of anterior cingulate atrophy than *C9orf72* carriers
Sha et al. (2012)[216]	Cross-sectional, retrospective, single-centre/ Structural: VBM	31 C9+ (15bvFTD, 11 FTD-MND, 2 ALS), 73 C9- (48	7.4	CDR-SB, IRI, phonemic fluency, semantic fluency, Delis Kaplan Executive Functioning Scale Design fluency test, TMT B test, series of similarities and proverbs, digit span test backward	- C9+ bvFTD vs C9-bvFTD: more delusions, greater impairment of working memory - C9+ FTD: more parietal and bilateral thalamic atrophy; C9-bvFTD: more medial frontal atrophy. - C9+FTD-MND: greater dorsal frontal and posterior cortical atrophy and cerebellar

		bvFTD, 19 FTD-MND, 6 ALS)			atrophy and right thalamic atrophy compared to C9- FTD-MND who had more ventral and temporal pole involvement.
Schonecker et al. (2018) [269]	Cross-sectional, prospective, single-centre/ Structural: atlas-based and mask-based volumetry, laterality indices	13 C9+, 45 sporadic (FTD or FTD/ALS), 19 HC	2.5	MMSE, FTD-CDR-SOB	- C9+: atrophy in sensory, premotor, posterior parietal, occipital, temporal and prefrontal subregions of thalamus. No cerebellar atrophy. - MRI volumetry of thalamus was of high predictive value in differentiating C9orf72 from sporadic FTD
McMillan et al. (2015) [229]	Longitudinal, prospective, single-centre/ Structural: GM density	20 C9+, 25 HC	3.2	MMSE, verbal call, letter fluency	- C9+: lower GM density in bilateral medial and lateral frontotemporal cortices, cerebellum, medial parietal cortex - Increased methylation associated with lower GM atrophy in right hippocampus, right thalamus, left premotor cortex - Faster GM atrophy with decreased C9orf72 methylation longitudinally
Mahoney et al. (2012) [260]	Longitudinal, retrospective, single-centre/ Structural: Cortical and	20 C9+, 15 HC	6.5	Weschler Adult Intelligence Scale, Weigl test, Stroop colour-word test, Hayling test, recognition memory test, Graded naming test, oldfield naming test, visual object and spatial	- C9+: greater mean rates of whole brain atrophy and ventricular expansion - C9+: symmetrical diffuse atrophy including thalamus and cerebellum

	subcortical regional volumes			perception battery, Graded difficulty arithmetic test, Baxter spelling tests	
Devenney et al. (2017) [263]	Cross-sectional, prospective, multi-centre/ Structural: VBM	14 symptomatic C9+, 42 symptomatic C9-, 23 HC	4.2	ACE-R, FTD-FRS, NPI, interview	- More likely to exhibit psychotic symptoms (delusions and hallucinations) - C9+: Increased psychotic symptoms correlated with atrophy in regions of the frontal, temporal and occipital cortices, thalamus, striatum and cerebellum - C9+: atrophy in bilateral anterior cingulate, dorsolateral and orbitofrontal prefrontal cortex, insular cortices, lateral parietal cortices, striatum, bilateral thalamus
Devenney et al. (2014) [267]	Cross-sectional, prospective, single-centre/ Structural: Visual rating scales	10 C9+ bvFTD, 19 C9- bvFTD, 35 HC	4.7	ACE-R, FTD-FRS, CBI, physical examination: aphasia, parkinsonism, apraxia, ataxia, eye movement abnormalities noted, Rey-Osttereith Complex figure test, visual object, space perception battery, Rey auditory verbal learning test, Rey-Osttereith complex figure test, Hayling test, FAS verbal fluency test, TMT, Sydney Language battery, test for reception of grammar, version 2	- C9+: less atrophy of orbitofrontal cortex, anterior cingulate, insula, and temporal pole - Slow progression - More likely to experience psychotic symptoms.
Convery et al.	Cross-sectional, retrospective, multi-centre/	26 C9+, 15 GRN,	5.5	n/a	- Smaller basal forebrain in C9+ compared to healthy controls

(2020) [272]	Structural: GIF parcellation method	15 MAPT, 83 HC			
Bocchetta et al. (2020) [237]	Cross-sectional, retrospective, multi-centre/ Structural: atlas propagation and label fusion	28 C9+, 18 GRN, 27 MAPT	4.8	n/a	- C9+: pulvinar atrophy, more lateral and medial geniculate nuclei involvement than *GRN* and *MAPT* carriers - All FTD subgroups shared mediodorsal nucleus atrophy
Bocchetta et al. (2018) [273]	Cross-sectional, retrospective, multi-centre/ Structural: atlas propagation and label fusion	29 MAPT, 18 GRN, 28 C9+, 97 HC	6	n/a	- C9+: greatest hippocampi atrophy in CA4, CA1, and dentate gyrus
Bocchetta et al. (2018) [270]	Cross-sectional, retrospective, multi-centre/ Structural: atlas propagation and label fusion approach	24 C9+, 15 GRN, 24 MAPT	5.6	n/a	- C9+: smallest thalami - ROC analysis showed a relatively poor ability to separate *C9orf72* from *MAPT* and *GRN* cases using thalamic volume
Bocchetta et al. (2016) [271]	Cross-sectional, retrospective, multi-centre/ Structural: atlas propagation and	17 C9+, 20 MAPT, 7 GRN, 18 HC	6	n/a	- C9+: smallest global cerebellar volume - C9+: lower volume in lobule VIIa-Crus I in superior-posterior regions of cerebellum and trend in lower volumes of interposed nuclei

	label fusion approach				
Bocchetta et al. (2015) [275]	Cross-sectional, prospective, single-centre/ Structural: optimised multimodal segmentation protocol	18 bvFTD (9 MAPT, 6 C9+, 3 nc), 18 HC	10.8	FDRS, CBI-R, MMSE	- C9+: trend in smaller anterior superior and left superior tuberal regions of hypothalamus.
Bede et al. (2018) [258]	Cross-sectional, prospective, single-centre/ Structural: volumetry, density analyses, CTh analyses, connectivity-based segmentation	26 FTD, 14 C9+ ALS-FTD, 12 C9- ALS-FTD, 36 ALSnci, 50 HC	2.4	ALSFRS-R, FrSBe, Stroop colour-word teat, Brixton Spatial anticipation test, backward digit span, category fluency, phonemic verbal fluency, local memory, verbal paired associate, auditory delayed recognition task, California Verbal learning tests, Rey-Osterrieth Complex figure	- C9+ ALS-FTD: preferential density reductions in thalamic sub-regions connected to motor and sensory cortical areas - C9- ALS-FTD: striatal pathology in subregions projecting to rostral-motor and executive cortical areas
Cistaro et al. (2014) [248]	Cross-sectional, prospective, single-centre/ PET/CT: Glucose metabolism	15 C9+ALS, 12 C9-ALS-FTD, 30 C9-	0.33	MMSE, FrSBe, HADS, Wisconsin card sorting test, trail making A and B, Stroop colour-word inference test, Wechsler memory scale-revised, letter and category fluency, Rey-Ostterith complex firgure test, token test, Wechsler adult intelligence	- C9+ ALS vs C9-ALS: hypometabolism in anterior and posterior cingulate cortex, insula, caudate and thalamus, left frontal and superior temporal cortex. Hypermetabolism in midbrain, bilateral occipital cortex, globus pallidus and left inferior temporal cortex

		ALS, 40 HC		scale revised, Raven's progressive coloured matrices	- C9+ALS vs C9-ALS-FTD: hypometabolism left temporal cortex
Van Laere et al. (2014) [246]	Cross-sectional, prospective, single-centre/ PET: Glucose metabolism, volume of interest and voxel-based analysis	70ALS (59 C9-, 11 C9+), 7 PLS, 4 PMA, 20 HC	1.3	ALSFRS-R	- Peri-Rolandic and variable prefrontal hypometabolism - C9+ ALS vs C9-ALS: relative hypometabolism in the thalamus and posterior cingulate
Diehl-Schmid et al. (2019) [278]	Cross-sectional, retrospective, multi-centre/ PET: Voxel-based approach, ROI analyses	22C9+, 22 C9-, 23 HC	4.54	MMSE, CDR, CERAD-NAB	- C9+ FTD: hypometabolism in thalamus bilaterally. No cerebellar hypometabolism
Castelnovo et al. (2019) [249]	Cross-sectional, retrospective, multi-centre/ PET: SPM voxel-based approach	3 C9+ALS, 2 C9+bvFTD, 1 C9+svFTD	2.5	Not available	- C9+bvFTD: prevalent frontal hypometabolism - C9+svFTD: right temporal polar and lateral involvement - C9+ALS: variable patterns of hypo- and hyper metabolism
Van der Burgh et al. (2020) [225]	Longitudinal, prospective, single-centre/ Structural: CTh analyses,	150 ALS, 156 HC	0.958	ALSFRS-R, ALS-FTD-Q,VFI, ECAS, FAB	- C9+ ALS: widespread GM and WM involvement at baseline and longitudinal WM changes in connectome

	subcortical volumetry; DTI: WM connectivity				- C9-ALS showed cortical thinning and loss of WM integrity confined to motor cortex
Van der Burgh et al (2019) [245]	Longitudinal, prospective, single-centre/ Structural: CSA; DTI: global probabilistic tractography	108 C9-ALS, 26 C9+ALS, 28 PLS, 56 PMA, 114 HC	0.958	ALSFRS-R	- C9+ALS: thinner segments at C2 and C3 levels at baseline. No longitudinal changes - ALS C9-: UCSC thinning at C1 to C4 at baseline and longitudinal changes in C3
Smith et al. (2019) [281]	Cross-sectional, prospective, single-centre/ Structural MRI/PET: standardised uptake value ratio at ROI and voxel level	6 svPPA, 6 C9+ bvFTD, 54 HC, 39 AD	3.16	n/a	- C9+ bvFTD: limited uptake in inferior frontal lobes in the ROI based analysis - No specific binding in svPPA or C9+ carriers on autoradiography
Rytty et al. (2014) [276]	Cross-sectional, prospective, single-centre/ Structural: VBM; rs-fMRI: spatial independent	7 C9+bvFTD, 8 HC	5.1	FBI-mod, VIQ, PIQ, digit span forward, digit span backward, immediate recall, delayed recall, visual memory, semantic fluency, phonemic fluency, TMT A and B, Rey figure copy	- Increased anti-correlation between bilateral thalamic parts of salience network and anterior sub-network of default mode network - Increased resting state connectivity in right-sided dorsal attention network

	component analysis				
Müller et al. (2020) [389]	Cross-sectional, prospective, single-centre/ DTI: Voxel-wise statistical comparison, tract-based analysis (FA maps) according to ALS stages	27 C9+ALS, 15 SOD1+ ALS, 32 HC	Not available	ECAS	- Both C9+ and C9-ALS: regional FA reductions along CST, in frontal and prefrontal brain areas - C9+: microstructural cortico-efferent involvement pattern
Ameur et al. (2016) [390]	Cross-sectional, retrospective, single-centre/ Structural: Severity of WM lesions using ad hoc 3-level visual score	17C9+ FTD, 11 GRN+FTD, 11 HC	Not available	n/a	- No WMH detected in C9+
Chipika et al. (2020) [236]	Cross-sectional, prospective, single-centre/ Structural: volumetry, shape deformation (vertex wise analysis and focal	100 ALS, 33 PLS, 117 HC	Not available	ALSFRS-R, ECAS	- C9+ ALS showed a trend of reduced mediodorsal-parateniual-reuniens volumes compared to C9- ALS. - C9+ALS displayed symmetrical pattern of thalamic atrophy in superior-inferior-posterior areas but showed more right thalamic involvement compared to C9-ALS

	density alterations ROI morphometry)				
Bocchetta et al. (2019) [274]	Cross-sectional, prospective, single-centre/ Structural: atlas based propagation and label fusion, volumetry	27 MAPT, 18 GRN, 29 C9+, 21 Tau, 39 TDP-43, 3 FUS, 107 HC	5.5	n/a	- *MAPT* had smallest AS in both hemispheres. - *C9orf72* and *GRN* had similar volume differences in most affected hemisphere, with greatest involvement in superficial group and accessory basal nucleus and lowest in lateral nucleus.
Lucidi et al. (2019) [279]	Cross-sectional, retrospective, single-centre/ structural MRI/PET: Global cerebral atrophy scale, MTA scale MTA, degree of leukoencephalopathy using the Fazekas scale	13 C9+FTD, 25 C9-bvFTD, 25 HC	Range: 1 – 12	ADL, IADL, NPI, interview, MMSE, FABit	- C9+ patients displayed more frontal atrophy than sporadic bvFTD - C9+ showed more widespread hypometabolism involving entire frontal, premotor, motor areas, striata and thalami
Chipika et al. (2020) [239]	Cross-sectional, prospective, single-centre/	100 ALS, 33 PLS,	Not available	ALSFRS-R, ECAS	- C9+: trend of total amygdala volume reduction (p=.055) - C9+: lateral nucleus (p=.043) and cortico-

	Structural: amygdala volumes, nucleus volumes, vertex analyses	117 HC			amygdaloid transition (p=.024) volume reduction
Mahoney et al. (2012) [261]	Longitudinal, retrospective, single-centre/ Structural: brain BSI, VBM, CTh analyses; DTI: TBSS	19 C9+, 22MAPT+, 24 GRN+, 1 VCP+	8.7	Symptoms at interview	- C9+FTD: extensive thinning of frontal, temporal and parietal cortices, subcortical GM in thalamus, cerebellum and WM changes in long intrahemispheric commissural and CST
Irwin et al. (2013) [265]	Longitudinal, retrospective, multi-centre/ Structural: GM density, CTh analysis	64C9+, 79 C9-	4	MMSE, letter-guided verbal fluency	- C9+FTLD vs C9- FTLD: greater atrophy in the right frontoinsular, thalamus, cerebellum and bilateral parietal regions

Table 2- 3 Selection of neuroimaging studies of symptomatic hexanucleotide carriers in *C9orf72*

2.3 The neuroradiology of upper motor neuron degeneration: PLS, HSP, ALS

The neuroimaging literature of motor neuron diseases is dominated by studies in ALS [391, 392]. There is a relative paucity of quantitative MRI studies in other motor neuron diseases despite the considerable disability, lack of disease modifying therapies and diagnostic challenges associated with most MNDs [380]. Radiological reports in primarily lateral sclerosis, Kennedy's disease, hereditary spastic paraplegia and spinal muscular atrophy are dominated by case series, and multimodal quantitative protocols have only been recently implemented in non-ALS MNDs [46, 234, 393].

In this edition of Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, Navas-Sánchez et al. present an intriguing study of motor cortex and corticospinal tract (CST) degeneration in hereditary spastic paraparesis type 4 (SPG4) [394]. The authors used a multiparametric imaging approach to evaluate pyramidal tract degeneration using both fixel-based analyses and probabilistic tractography. The authors identify clinico-radiological correlations and detect inferior-predominant CST degeneration. The study offers multiple learning points potentially relevant to other MNDs. While correlations between CST metrics and motor disability are confounded by co-existing lower motor neuron degeneration in ALS [395], these are pertinent to upper motor neuron predominant disorders such as PLS and HSP [381]. Fixed-based analysis (FBA) is a relatively novel framework which offers integrity indices for single fibre populations and permits the evaluation of crossing fibres. White matter degeneration in MND is typically evaluated by tract-based methods [252], template-based approaches [396], or tractography [232] which makes the appraisal of crossing fibres challenging. Non-Gaussian diffusion protocols, such as diffusional kurtosis imaging (DKI), q-space imaging (QSI) or neurite orientation dispersion and density imaging (NODDI) have only been recently applied to MND datasets and have already contributed important insights [57, 293, 294]. Furthermore, CST changes in MND are often preferentially assessed in the posterior limb of the internal capsule, and the segmental profile of the pyramidal tracts are seldom systemically characterised from the superior corona radiata to the spinal cord [58, 242, 360, 384].

A number of radiological cues are associated with HSP on standard clinical imaging, such as thinning of the corpus callosum, spinal cord cross-sectional area reduction, ventricular enlargement and periventricular T2/Flair signal hyperintensities. Characteristic bilateral signal change in the forceps minor in SPG11/SPG15 has been referred to as the "ear-of-the-lynx sign" which is best seen on axial views at the frontal horn of the lateral ventricles, but the specificity of qualitative cues in MND is contentious [69]. Corpus callosum thinning and forceps minor degeneration are also commonly observed in other motor neuron diseases [397].
Cortical change in SPG4 has been previously investigated by voxel based morphometry [398] and cortical thickness analyses [399]. White matter degeneration in SPG4 has been evaluated by tract-based special statistics (TBSS) [398], voxel-based FA analyses [400], and tractography [399]. A consensus finding of these studies is that CST changes are more readily detected than the more elusive primary motor cortex changes. In addition to standard grey and white matter techniques, a variety of volumetric approaches [400], thalamus imaging [401], resting-state functional MRI [402], magnetic resonance spectroscopy [403] and spinal cord morphometry [399] have been applied in SPG4. PET studies often capture metabolic changes beyond the motor cortex including the involvement of frontotemporal regions [404]. Despite the sample size limitations and the divergent methodologies of existing SPG4 studies, pyramidal tract degeneration, motor cortex thinning, thalamus atrophy, cerebellar involvement and frontotemporal changes seem relatively consistent observations.

One of the main drawbacks of single-phenotype studies is that imaging findings are identified based on comparisons to healthy controls and the identified patterns are often interpreted as a "signature" of the cohort. The specificity of these findings however can only be ascertained if multiple phenotypes and disease controls are also included in comparative analyses. This is a common challenge of MND imaging, where low-incidence phenotypes often show similar anatomical patterns of degeneration. Cerebral changes in PLS for example are difficult to distinguish from ALS; both exhibiting CST, corpus callosum, cerebellar and some degree of frontotemporal change [205, 405, 406]. Only the departure from "single group versus controls" study designs and the inclusion of several relevant cohorts will permit the comparative characterisation of imaging traits and enable the definition of phenotype-specific signatures. Distilling phenotype-specific

features can then be utilised in classification algorithms to aid the categorisation of single subjects [377, 382].

Imaging initiatives across the spectrum of MNDs offer invaluable learning opportunities and resourceful imaging protocols can be readily adopted and developed to be utilised in other MND phenotypes. Concepts in study design and data interpretation frameworks are also largely transferrable. Advances in academic imaging in MND are likely to gradually filter down to pragmatic clinical and pharmaceutical trial applications.

3 Structural alterations in cerebral motor areas of poliomyelitis survivors

3.1 Introduction

Poliomyelitis was one of the most debilitating acute viral infectious diseases of the 20th century before the availability of the polio vaccine [407]. It predominantly affected children under 5 years of age, causing flaccid paralysis in less than 1% of those infected and carried a mortality rate of 5-10% due to respiratory failure [408]. Despite being 99% eradicated globally today, there are currently 15-20 million people living with the lasting sequelae of the disease [2], of which 20-85% are experiencing post-polio syndrome (PPS) [4, 409].

Post-polio syndrome was first described by French neurologists Raymond and Jean-Martin Charcot in 1875 [3, 410]. It is a slowly progressive lower motor neuron condition that can affect polio survivors after a considerable period of functional stability long after the acute poliomyelitis infection [62, 63]. PPS typically manifest as new or progressive asymmetrical muscle weakness, atrophy, fatigability, myalgia or fasciculations [78, 105], but also as chronic pain, dysphagia, dysphonia or in some cases as respiratory weakness [79, 80]. Despite being traditionally regarded as a pure lower motor neuron syndrome, non-motor symptoms such as generalised fatigue [411], cold intolerance, neuropsychological deficits [84], sensory impairment [82] and sleep disorders [87, 91, 93, 97] have also been described, all of which have considerable impact on the patient's well-being and quality of life [89, 412, 413]. Research studies in PPS invariably focus on lower motor neuron and muscle pathology utilising quantitative neurophysiological techniques [72, 414], muscle biopsies [30, 41] and wet biomarkers [37, 43]. Despite clinical observations suggestive of cerebral pathology there is a striking paucity of imaging and post mortem studies and account of cerebral involvement are conflicting. While early post mortem studies in the 1940s identified widespread cerebral pathology involving the reticular formation, posterior hypothalamus, thalamus, putamen, caudate, locus coeruleus, substantia nigra as well as preferential involvement of the precentral gyrus and pre-motor areas [49, 50, 52], more recent studies did not confirm these observations [59] and conversely recent neurophysiological studies detected changes suggestive of a compensatory functional reorganisation of the motor cortex [415, 416].

From a clinical perspective, PPS remains a diagnosis of exclusion and the March of Dimes diagnostic criteriais typically applied [62, 63]. Despite coordinated research efforts, there aetiology is not convincingly established, the underlying pathophysiology is not confirmed [417], and no validated prognostic and monitoring markers currently exist. The mainstay of management in PPS comprises symptomatic relief [93], prevention of further deterioration through conservative measures [129], energy conservation strategies [130, 131], muscle training [134], and improving cardiovascular fitness [141, 148, 150]. Past clinical trials for potential disease-modifying drugs have shown disappointing results with limited effects [183-185, 188, 190], with the exception of intravenous immunoglobulin (IVIg) therapy which proved promising [196].

Unlike the imaging profile of other motor neuron diseases [391] such as amyotrophic lateral sclerosis (ALS) [418], primary lateral sclerosis [381] or SBMA[393], the radiological correlates of PPS are woefully understudied. Thanks to advances in magnetic resonance imaging (MRI), phenotype-specific grey matter and white matter alterations can be reliably characterised *in vivo*. The evaluation of cerebral changes in PPS not only helps to explore the pathological substrate of common PPS-associated symptoms but more broadly, adds to the existing literature of LMN-predominant MNDs [69].

Brain imaging in other slowly progressive LMN-predominant MNDs have consistently shown a degree of cerebral reorganisation. Increased grey matter density with underlying neuronal reorganisation was observed in both adult onset spinal muscular atrophy (SMA) [419] and LMN-dominant ALS [367], which has been interpreted as a compensatory mechanism. Cortical reorganisation and adaptive functional changes were also observed in cases of limb amputation [420] or spinal cord injury [421] after longstanding motor training [422]. The main objective of this study is the comprehensive characterisation of grey and white matter alterations in PPS in contrast to healthy controls and a cohort of MND patients with ALS.

3.2 Methods

3.2.1 Participants

Thirty-six patients with PPS, 88 patients with ALS and 117 healthy controls (HC) were included in a prospective neuroimaging study. The study was approved by the Ethics

(Medical Research) Committee - Beaumont Hospital, Dublin, Ireland, and all participants provided informed consent prior to inclusion. Participating PPS patients were diagnosed according to the March of Dimes diagnostic criteria [62, 63] and ALS patients had 'probable' or 'definite' ALS according to the El Escorial criteria. Inclusion criteria included the ability to tolerate the duration of MR imaging and exclusion criteria included comorbid neuro-inflammatory, neurovascular, neoplastic, psychiatric condition or prior head or spinal cord injuries. The healthy controls were unrelated to participating patients and had no known neurological or psychiatric diagnoses. Demographic and clinical details were carefully recorded for all PPS patients, including age of acute poliomyelitis, age of PPS onset, handedness, age, education and the relevant demographic variables were used as covariates in statistical models. PPS and ALS patients also underwent standardised clinical evaluation, and the revised ALS functional rating scale (ALSFRS-r) was administered to appraise functional disability. ALSFRS-r subscores were individually recorded to evaluate bulbar, upper limb, lower limb and respiratory dysfunction. Muscle strength was assessed using the Medical Research Council (MRC) scale and composite scores for left and right, upper and lower limbs were individually calculated. Upper limb score comprised of shoulder abduction and adduction, elbow flexion and extension, wrist flexion and extension and finger flexion and extension. Lower limb score was calculated using hip flexion and extension, knee flexion and extension and ankle dorsiflexion and plantarflexion. ALS patients were screened for ALS-associated mutations and the presence of GGGGCC repeat expansions in *C9orf72*. As described previously [423], the presence of the *C9orf72* hexanucleotide repeat expansion was determined using repeat-primed polymerase chain reaction (PCR) as described previously [214]. As the ALS group was included as a motor neuron disease (MND) control group, only *C9orf72* negative patients were included to reduce radiological heterogeneity.

3.2.2 Neuroimaging methods

A 3 Tesla Philips Achieva system was used with an 8-channel receive-only head coil to acquire magnetic resonance (MR) data. A standardised imaging protocol was implemented. A 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) sequence was utilised to acquire T_1-weighted images with a spatial resolution of 1 mm^3, field-of-view (FOV) of 256 x 256 x 160 mm, flip angle = 8°, SENSE factor = 1.5, TR/TE = 8.5/3.9 ms, TI =1060 ms, and an acquisition time of 7 min 30 s. A spin-echo

echo planar imaging (SE-EPI) sequence was implemented to acquire diffusion tensor images (DTI) with a 32-direction Stejskal-Tanner diffusion encoding scheme. Spatial resolution = 2.5 mm^3, FOV = 245 x 245 x 150 mm, 60 slices acquired with no interslice gap, TR/TE = 7639 / 59 ms, SENSE factor = 2.5, b-values = 0, 1100 s/mm^2.

3.2.3 Total intracranial volume estimation

TIV was estimated by linearly aligning each participant's skull-stripped brain to the MNI152 brain image in MNI space, the inverse of the determinant of the affine registration matrix was calculated and multiplied by the size of the template. FSL-FLIRT [424] was used for registration to template and tissue type segmentation was performed using FSL-FAST [425, 426].

3.2.4 Whole-brain imaging analyses

Grey matter alterations in PPS were first evaluated by morphometric analyses using the FMRIB's FSL suite [427, 428]. Pre-processing included standard steps such as skull-stripping (BET), motion-corrections and tissue-type segmentation. Using affine registration, individual grey-matter partial volume images were aligned to the MNI152 standard space. A study-specific GM template was created to which the individual grey matter images were non-linearly co-registered. Permutation based non-parametric inference was used for group comparisons, with the threshold-free cluster enhancement (TFCE) method [429]. Comparisons of patient groups and controls were corrected for age and gender and total intracranial volumes (TIV). Tract-based spatial statistics were utilised to assess white matter changes over the entire brain. Subsequent to skull removal and eddy current corrections, a tensor model was fitted to the raw diffusion data to generate maps of fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD). FMRIB's image analysis suite was utilised for non-linear registration and skeletonisation of each subject's images [430]. FA, AD and RD images were compiled into a single 4D image file and a mean FA mask was created. Similar to grey matter analyses, permutation-based non-parametric inference was used for the voxel-wise analysis of diffusivity parameters using design matrix-defined contrasts which included group membership, age, and gender. The threshold-free cluster enhancement (TFCE) method was implemented and resulting statistical maps were thresholded at $p < 0.05$ TFCE family-wise error (FWE).

3.2.5 Region of interest analyses

To further explore the regional grey matter profile of PPS, supplementary morphometric analyses were carried out in the precentral gyrus, brainstem, Broca's area, cerebellum, frontal lobe, occipital lobe, orbitofrontal cortex, parietal lobe, precentral gyrus, temporal lobe, and Wernicke's area. The labels of the Harvard-Oxford cortical probability atlas [431] and the MNI probability atlas [432] were used to define grey matter regions of interest. Average grey partial volume (matter intensity) values were retrieved from the above regions and post hoc statistics were performed. The volume profiles of bilateral structures were averaged between left and right. Assumptions of linearity, normality and homogeneity of variances were checked and analyses of covariance (ANCOVAs) were performed with study group membership (PPS, ALS or HC) as the independent variable, regional grey matter volumes as dependent variables. Age, gender, education and TIV were included in the model as covariates. A p-value of < 0.05 was considered significant. Estimated marginal means of region grey matter values, standard error, between-group ANCOVA significance and post hoc group contrasts were summarised in a dedicated table.

3.3 Results

3.3.1 Clinical profile

The demographic profile of controls, PPS and ALS patients is presented in **Table 3-1**. Even though patients and controls were matched in key demographic variables, statistical models for imaging indices included age, gender and education as covariates.

	HC ***n*=117**	**PPS** ***n*=36**	**ALS C9-** ***n*=88**	***P* value**
Age (years)	63.38 (11.914)	66.97 (5.629)	60.18 (10.311)	.052
Gender (male)	56 (47.9%)	16 (44.4%)	56 (63.6%)	.059
Education (years)	13.31 (3.294)	12.56 (3.691)	13.58 (3.169)	.064
Handedness (right)	109 (93.2%)	34 (94.4%)	81 (92%)	.855

Table 3- 1 The demographic profile of study participants.

The mean age at initial acute poliomyelitis infection was 33.50 (*SD*=37.867) months old, younger than 3 years of age. At the initial paralytic poliomyelitis infection, all patients were affected in their lower extremities and 27.7% of patients were also affected in the upper limbs. Three PPS patients (8.3%) had bulbar involvement at the initial infection and 2 patients had respiratory weakness spending a period of time in the 'iron lung'. Polio patients spent a mean of 2.68 (*SD*=2.757) years in hospital during their childhood and underwent an average of 2 surgeries (Range: 0 to 10) including corrective osteotomies to shorten a leg, leg-lengthening surgeries using the Ilizarov external fixation technique [433], muscle and tendon transfers, arthrodesis, and tendon release surgeries. More than half of the post-polio syndrome patients (61.1%) included in this study had leg length discrepancy ranging from 0.5 cm to 8 cm. The mean age of onset of PPS was 55.08 (*SD*=9.075) years and the mean period between the initial infection and PPS onset was 52.29 (*SD*= 9.917) years. All of the included PPS patients experienced new weakness in their lower limbs; 63% only in their lower limbs while the rest (36.1%) also reported new

weakness in their upper limbs. Two-thirds reported weakness in their originally affected body region, while up to 30.6% also reported weakness in previously unaffected limbs. Neurological examination revealed lower limb predominant weakness (LL right: *M*=23.528, *SD*=7.3959; LL left: *M*=24.306, *SD*= 5.9453, UL right: *M*=39.778, *SD*= 2.4479; UL left: *M*= 37.958, *SD*= 3.7690). As expected in a LMN condition, none of the PPS patients had spasticity and the mean total Penn UMN burden score was only 1.81 (*SD*= 2.424). Consistent with the physical examination, marked disability was noted in the lower limbs (*M*=6.78, *SD*= 1.514) while the upper limbs were less affected. The majority of patients (88.9%) were able to ambulate and only 4 PPS patients were wheelchair bound. Of those able to mobilise, 22.2% did so without any walking aids while the rest used custom-made shoes and orthotics (44.4%), walking stick (41.7%) or 2 crutches or stroller (13.9%). Up to a third of PPS patients experienced a fall in the preceding 12 months period (33.3%). The majority of patients were able to drive, but more than half (52.8%) had to make modifications to their cars and 2 patients had to give up driving due to PPS. Six of the included PPS patients were still working at the time of the study. The majority of patients retired at a mean age of 61.69 years (*SD*=6.856) (Range: 43 to 74), and only 4 patients (13.3%) had to retire precociously due to PPS. Bulbar and respiratory function was relatively spared in PPS patients. None of the patients required a feeding tube and only 3 patients used NIV at night. The clinical profile of PPS patients is presented in **Table 3-2**.

	Mean (SD)
Bulbar (max 12)	11.36 (1.199)
Upper limb (max 12)	10.50 (1.732)
Lower limb (max 12)	6.78 (1.514)
Respiratory (max 12)	11.36 (1.313)
Total ALSFRS-R score	40.00 (3.986)
MRC – Right upper limb (max. 40)	39.778 (2.4479)
MRC – Left upper limb (max. 40)	37.958 (3.7690)
MRC – Right lower limb (max. 30)	23.528 (7.3959)
MRC – Left lower limb (max. 30)	24.306 (5.9453)
Symptom duration (months)	148.11 (100.354)
Age at acute poliomyelitis (months)	33.50 (37.867)
Age at PPS onset (years)	55.08 (9.075)
Time between acute poliomyelitis to PPS onset (years)	52.29 (9.917)
New symptoms	**Proportion of patients (%)**
Weakness	36 (100%)
Decreased endurance	29 (80.6%)
Pain	28 (77.8%)
Cold intolerance	33 (91.7%)
Fatigue	29 (80.6%)
Hypersensitivity	2 (5.6%)
Bulbar involvement	12 (33.3%)
Respiratory difficulties	8 (22.2%)
Poor sleep	19 (52.8%)
Breathing related sleeping disorders	5 (13.9%)

Table 3- 2 The Clinical profile of the post-polio cohort, Symptoms experienced by PPS patients

3.3.2 Grey matter findings

On grey matter morphometric analyses increased regional volumes were identified in the PPS cohort in lingual, peri-calcarine, cerebellar and brainstem regions compared to healthy controls at $p < 0.05$ FWE TFCE. In comparison to ALS patients, PPS patients exhibited higher partial volumes values in the bilateral motor cortex, mesial temporal lobes, cerebellum and occipital lobe. (**Figure 3-1**.)

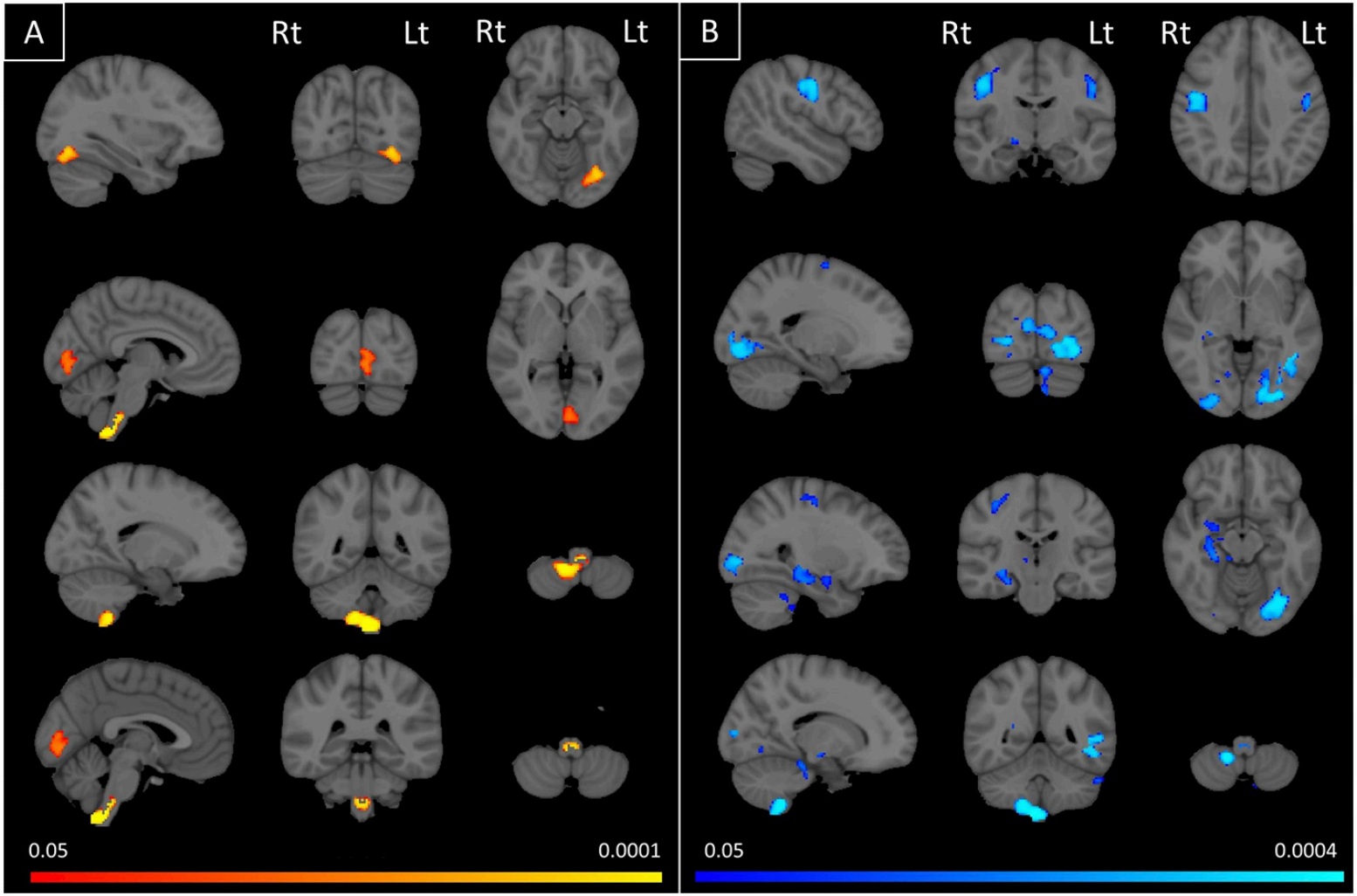

Figure 3- 1 Increased grey matter density in PPS compared to HC (A) and ALS patients (B). Contrast corrected for age, gender, education and total intracranial volumes, statistical maps shown with corresponding colour bars using *p* values corrected for FWE. Radiological convention used.

Our whole-brain VBM-type analyses were complemented by ROI analyses. Increased grey matter density was noted within the brainstem (p=.001), cerebellum (p= .033) and occipital lobe (p=.028) compared to healthy individuals, but no differences were observed in primary motor cortex. No cerebral grey matter changes were noted in the PPS cohort in frontotemporal regions including the orbitofrontal cortex, Brocas's or Wernicke's areas. Compared to the ALS group, increased grey matter volumes were noted in the brainstem, frontal lobe, occipital lobe and precentral gyrus. (**Table 3-3**.)

Grey matter ROIs	Study group	Estimated Marginal Mean	Standard error	MANCOVA Sig. (*p*)	PPS vs HC	PPS vs ALS C9-	ALS C9- vs HC
Brainstem	HC	0.080640	0.000660	**.001***	**.001***	**.024***	.547
	PPS	0.085831	0.001215				
	ALS	0.081990	0.000754				
Cerebellum	HC	0.490554	0.004158	**.033***	**.033***	**.069^{t}**	1.000
	PPS	0.513279	0.007659				
	ALS	0.492599	0.004754				
Frontal lobe	HC	0.454041	0.002223	**.001***	1.000	**.020***	**.002***
	PPS	0.455585	0.004094				
	ALS	0.442407	0.002541				
Occipital lobe	HC	0.448231	0.002770	**.002***	**.028***	**.001***	.507
	PPS	0.463694	0.005103				
	ALS	0.442380	0.003167				
Orbitofrontal cortex	HC	0.494966	0.003729	.107	1.000	.811	.112
	PPS	0.491949	0.006868				
	ALS	0.483008	0.004263				
Parietal lobe	HC	0.429299	0.002259	.082	.918	.112	.373
	PPS	0.434240	0.004161				
	ALS	0.423963	0.002583				
Precentral gyrus	HC	0.465694	0.002848	**<.001***	1.000	**.004***	**<.001***
	PPS	0.460406	0.005245				
	ALS	0.440441	0.003256				
Temporal Lobe	HC	0.505666	0.002767	**.008***	1.000	.287	**.007***
	PPS	0.502658	0.005096				
	ALS	0.492612	0.003163				

Table 3- 3 The regional grey matter profile of PPS, HC and ALS patients**.** Group comparisons are corrected for age, gender, education and transcranial volumes and are Bonferroni corrected. Statistically significant *p*-values are marked with asterisk* and trends with a 't'

3.3.3 White matter findings

Three diffusivity metrics were evaluated, fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD). Increase FA was noted in PPS compared to healthy controls along the entire cerebral course of the corticospinal tracts from the precentral gyrus, through the internal capsule to the mesencephalic crura. Additionally, higher FA was noted in the cerebellum, bilateral mesial temporal lobes and inferior frontal brain regions. The contrast to the ALS group was even more striking capturing higher FA in nearly all major commissural bundles and association tracts, including the corpus callosum and the fornix. (**Figure 3-2**.)

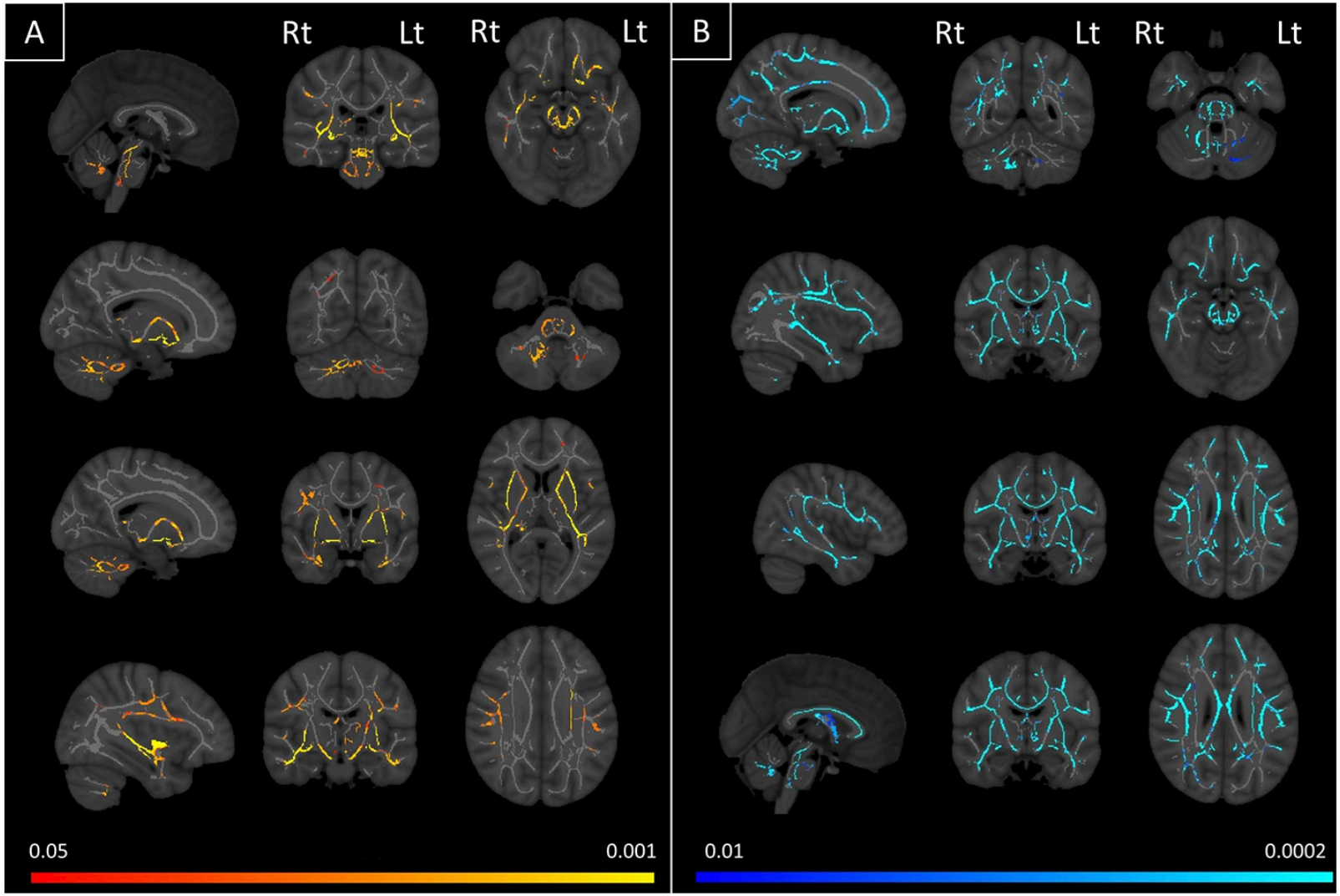

Figure 3- 2 Regions of increased fractional anisotropy in PPS compared to HC (A) and ALS patients (B). Contrasts are corrected for age, gender and education. Radiological convention used.

Higher AD was identified in the PPS cohort compared to healthy controls along the entire cerebral course of the corticospinal tracts, fornix, corpus callosum, mesial temporal lobes and brainstem. (**Figure 3-3A**) Significantly higher AD was detected in nearly the entire white matter skeleton in PPS patients compared to ALS patients. (**Figure 3-3B**) Lower radial diffusivity (RD) was observed in the PPS group compared to healthy controls in cerebellum and external capsule. (**Figure 3-3C**) Lower RD was detected in PPS in contrast to ALS along the bilateral pyramidal tracts, corpus callosum, cerebellum and mesial temporal lobes. (**Figure 3-3D**)

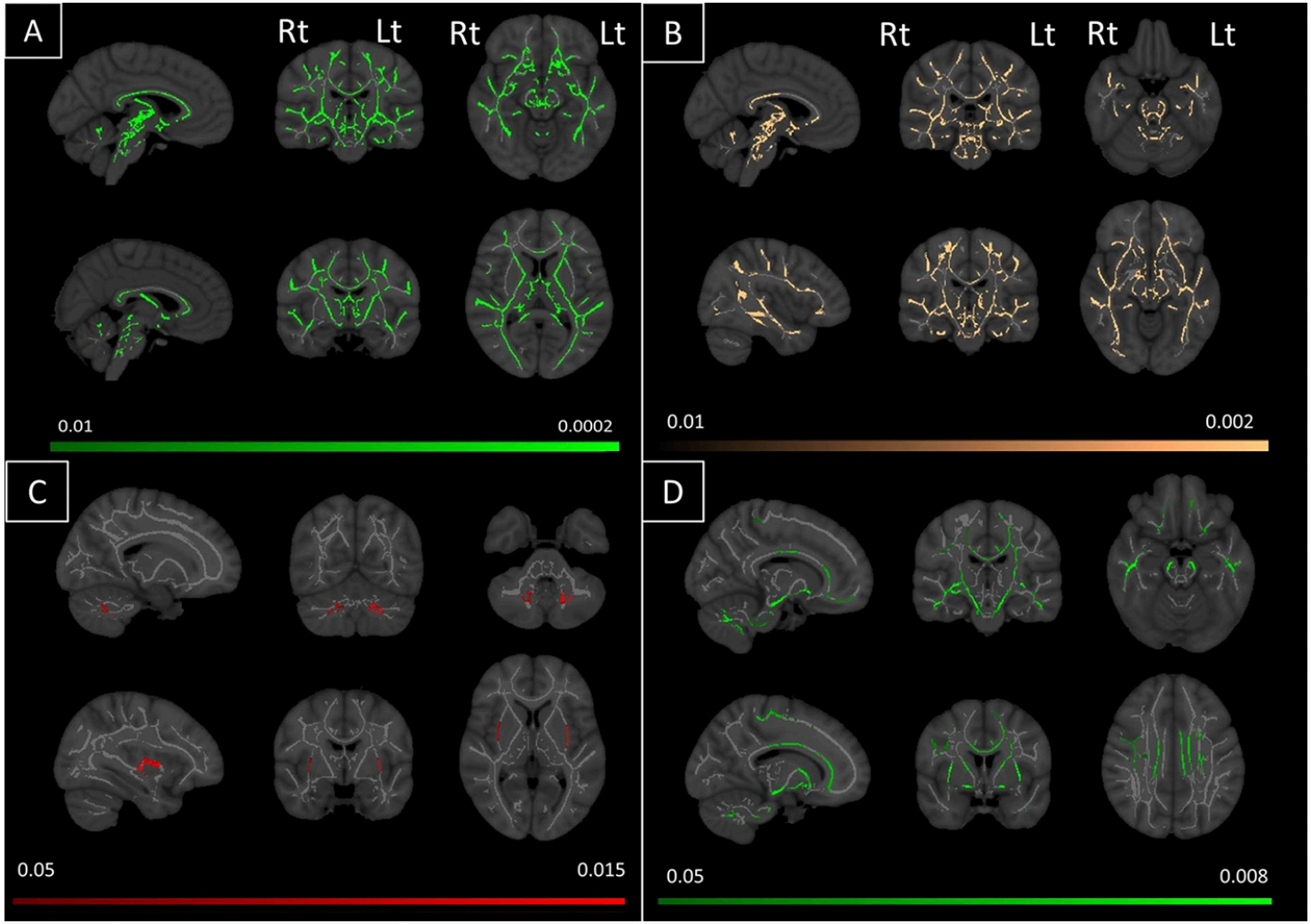

Figure 3- 3 The axial diffusivity profile of PPS in comparison to HC (A) and ALS patients (B). The radial diffusivity profile of the PPS cohort in contrast to HC (C) and ALS patients (D). Statistical maps are shown according the colour bars. *P*-values are corrected for age and gender. Radiological convention used.

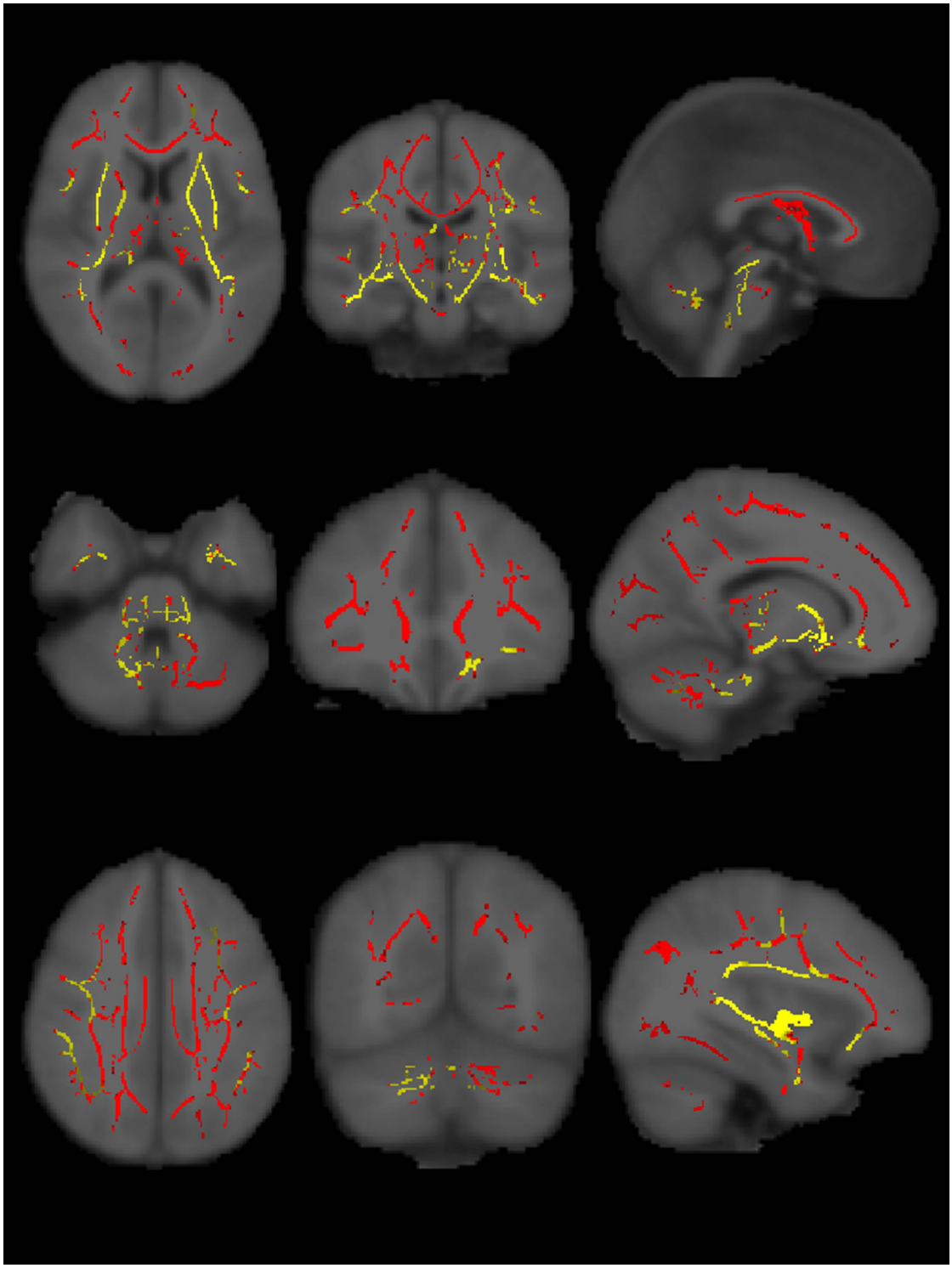

Figure 3- 4 A synthesis of overlapping anatomical patterns of increased FA in PPS both in comparison to healthy controls and patients with ALS is indicated in yellow colour ($p < 0.05$ TFCE corr.). The regions include bilateral corticospinal tracts, brainstem white matter, bilateral mesial temporal white matter regions, anterior limb of the internal capsule and external capsules in both hemispheres in a relatively symmetric distribution, as well as a left orbitofrontal focus. Additional anatomical areas of increased FA in PPS only identified in contrast to patients with ALS are highlighted in red colour ($p < 0.05$ TFCE corr.). These areas include the fornix, corpus callosum, cingulate, intra-thalamic, left cerebellar regions and widespread frontal, parietal and occipital white matter regions.

3.3.4 Data description

The raw grey and white matter profiles of the study groups are presented in **Figure 3-6 and Figure 3-6** as box plots. Additionally, demographically adjusted estimated marginal means are presented for key grey and white matter variables in **Figure 3-7 and 3-8**. The comparative profile of study groups with references to healthy individuals are illustrated by radar plots to further highlight regional grey (**Figure 3-9**) and white matter alterations. (**Figure 3-10**) Percentage change in grey matter partial volumes were calculated based on the estimated marginal means corrected for age, gender, education and total intracranial volumes. Percentage change in white matter diffusivity metrics were based on the estimated marginal means corrected for age, gender and education.

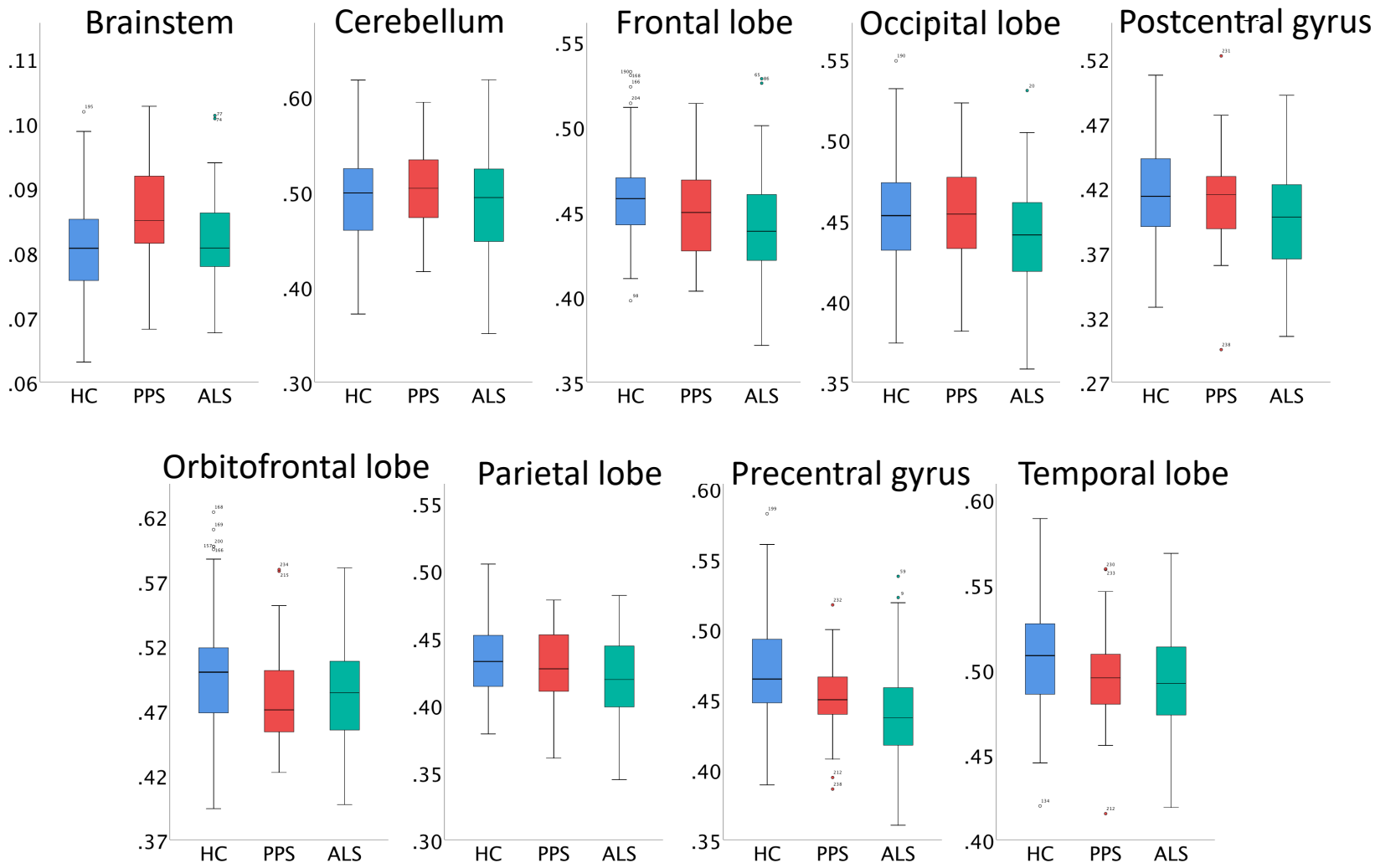

Figure 3- 5: The comparative regional partial volume profile of PPS, ALS and HC.

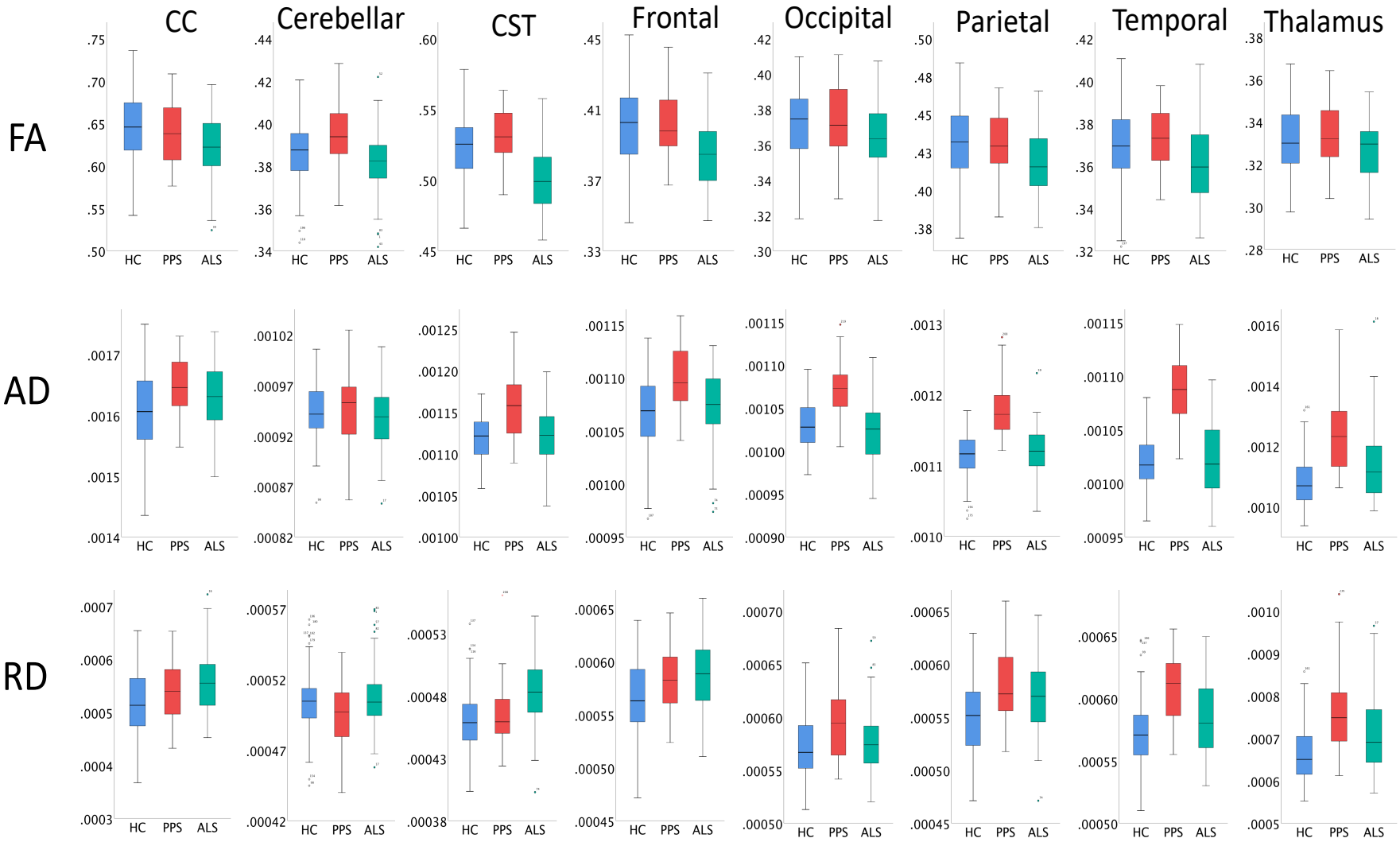

Figure 3- 6 The comparative raw white matter region-of-interest profiles of PPS, ALS and HC.

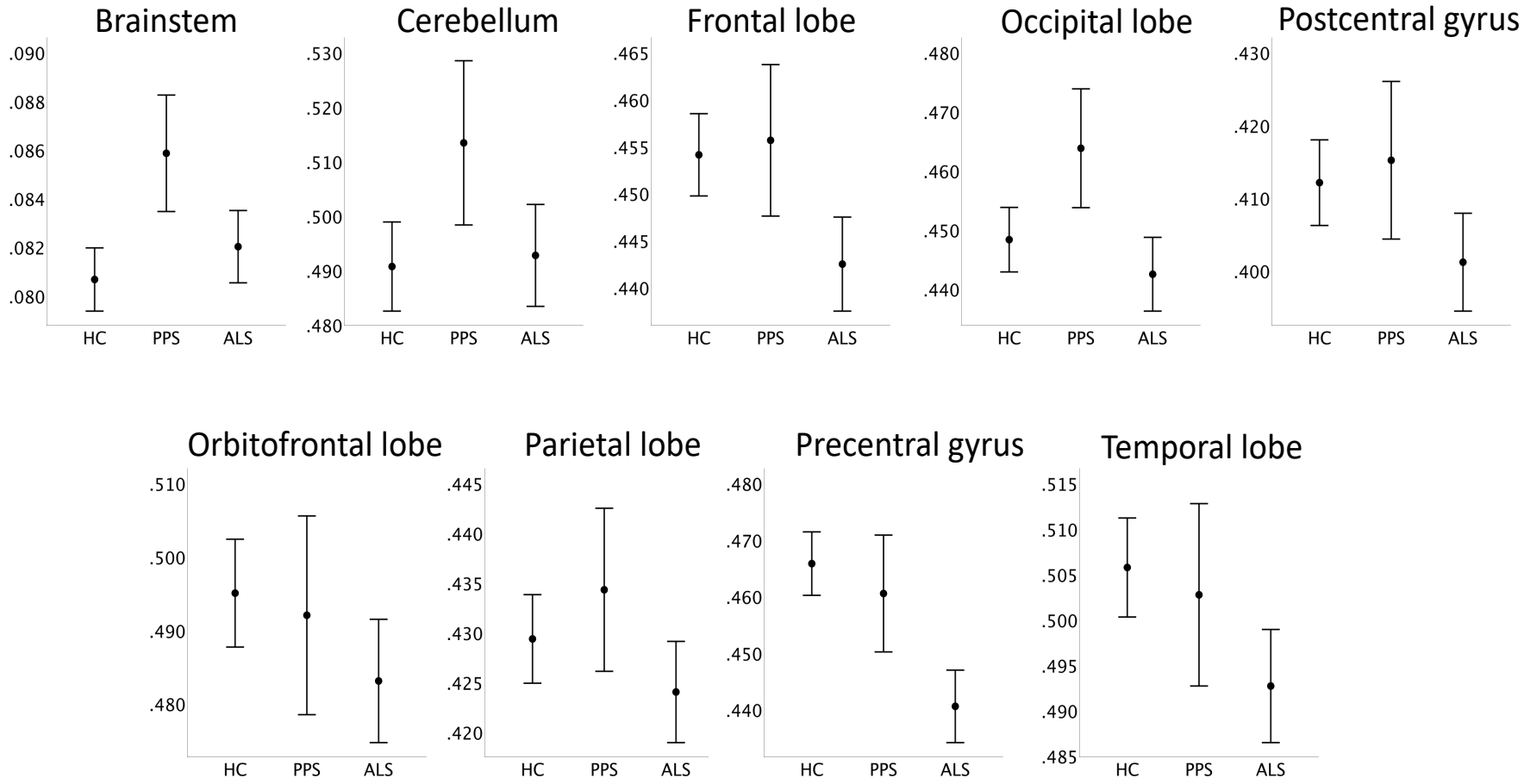

Figure 3- 7 The volumetric profile of HC, PPS and ALS based on estimated marginal means adjusted for the following values: Age = 59.84, Gender = 1.47, Education = 13.78, TIV = 1,424,021.23. Error bars represent 95% confidence interval.

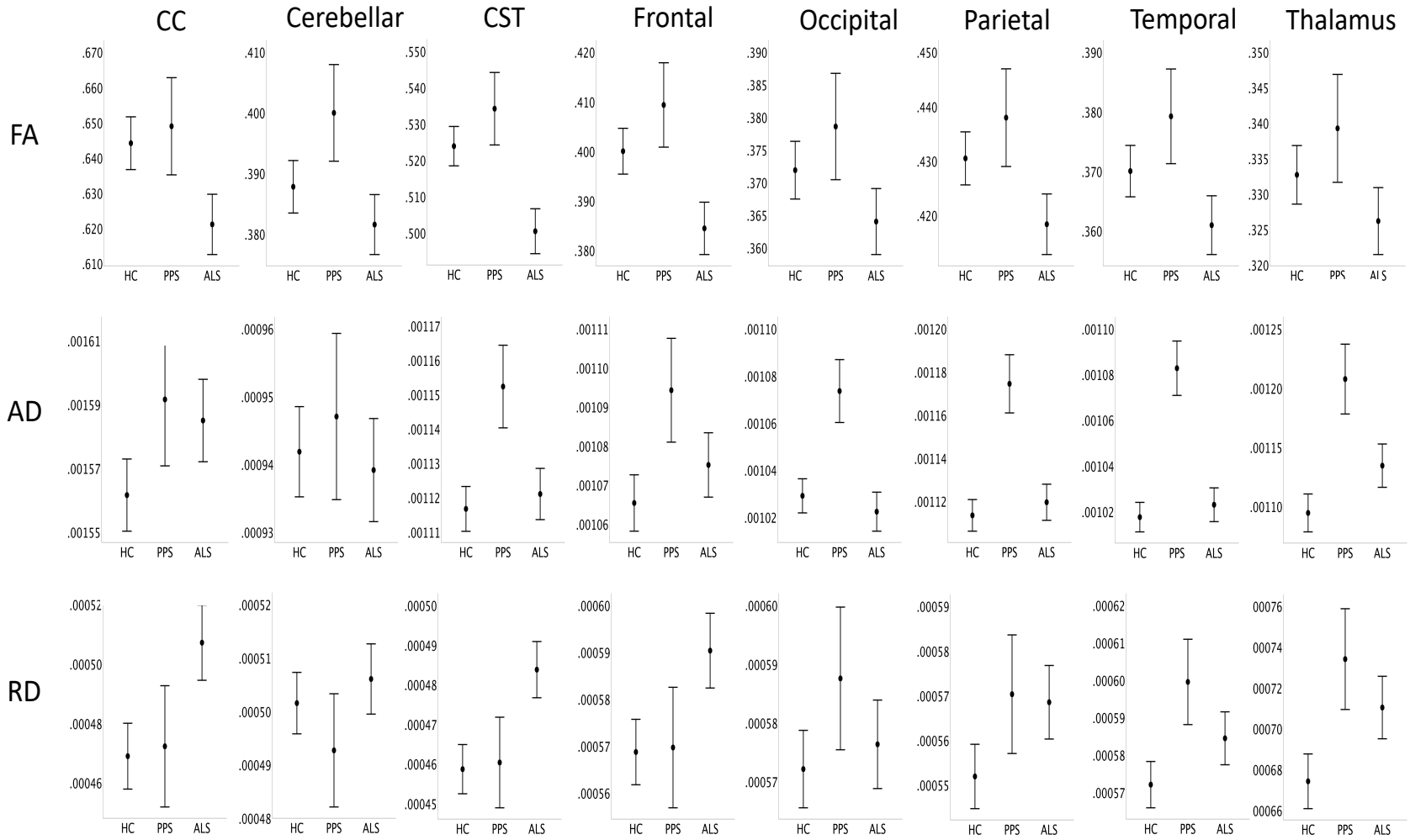

Figure 3- 8 The diffusivity profile of HC, PPS and ALS based on estimated marginal means adjusted for the following values: Age = 59.84, Gender = 1.47, Education = 13.78. Error bars represent 95% confidence interval.

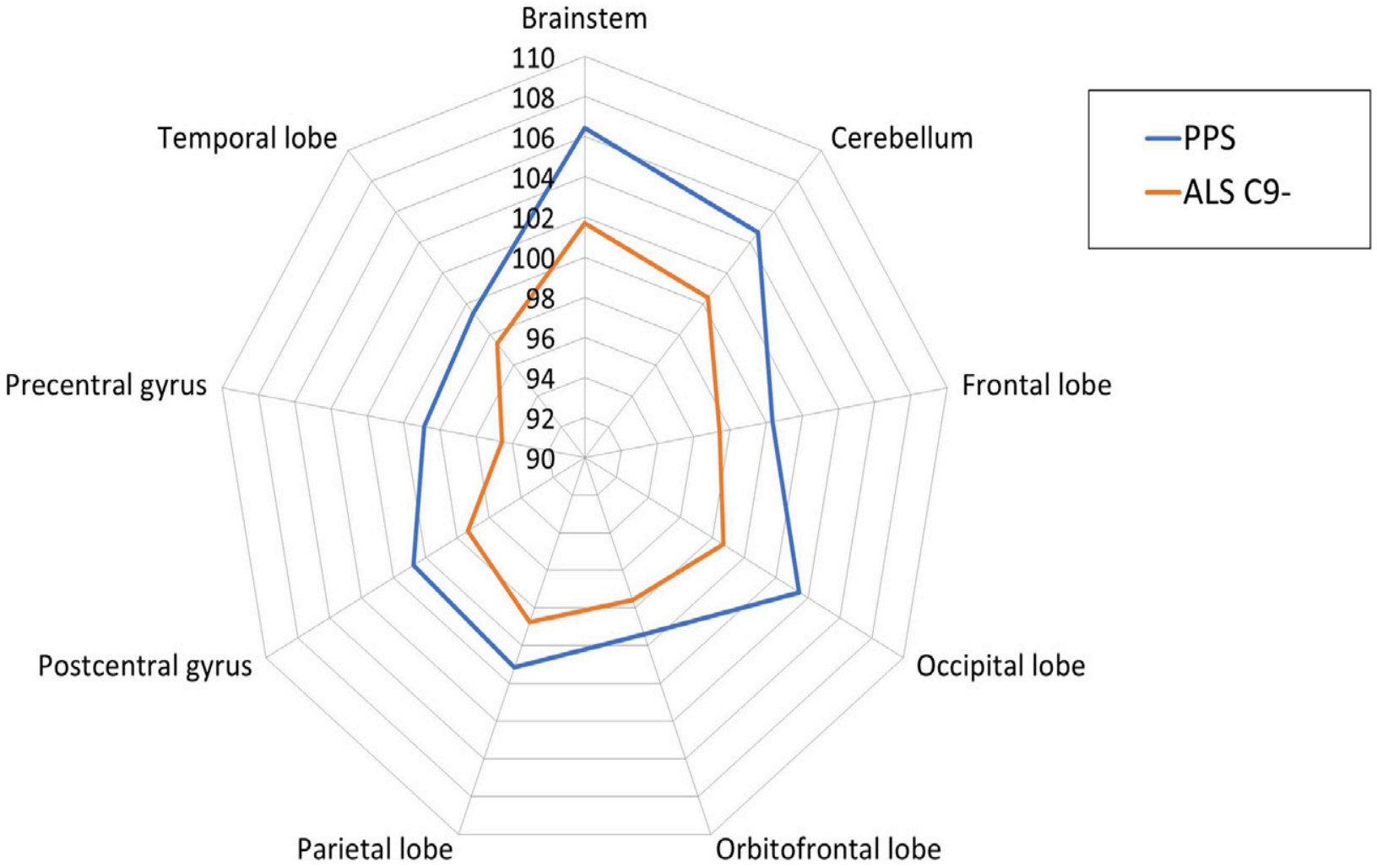

Figure 3- 9 Radar plot illustrating the grey matter ROIs profile of PPS and patients with ALS with reference to HC. 100% represents the estimated marginal means of healthy controls for each grey matter ROI. Estimated marginal means of volumes were calculated with the following values: Age = 59.84, Gender = 1.47, Education = 13.78, TIV = 1,424,021.23.

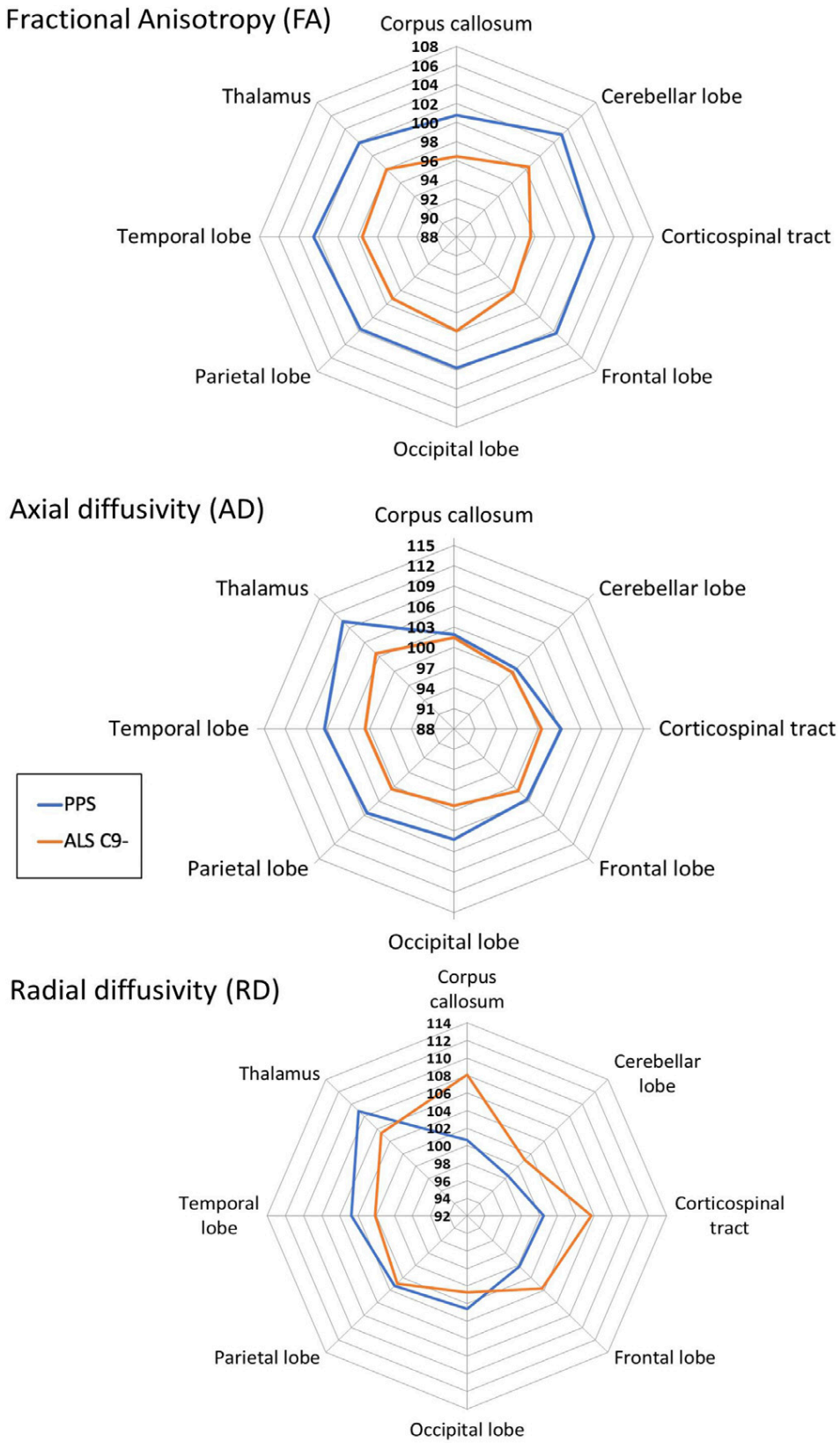

Figure 3- 10 Radar plot illustrating the diffusivity profile of poliomyelitis survivors (PPS) and patients with amyotrophic lateral sclerosis (ALS) with reference to healthy controls. 100% represents estimated marginal means of healthy controls for each structure. Estimated marginal means of the relevant metrics were calculated with the following values: Age = 59.84, Gender = 1.47, Education = 13.78.

3.4 Discussion

The overarching objective of this study was to characterise cerebral changes in post-polio syndrome, which has been previously debated in the literature [49, 59, 415]. Our imaging findings revealed no evidence of cortical and subcortical grey matter atrophy, but conversely, revealed focal areas of grey matter hypertrophy compared to controls involving the brainstem, cerebellar and occipital regions. This pattern of cortical reorganisation was accompanied by increased white matter tract integrity in the corticospinal tracts, cerebellum, bilateral mesial temporal lobes and inferior frontal tracts. The very same anatomical regions which showed degeneration in ALS, revealed increased integrity in the PPS cohort.

The structural and diffusion profile observed in PPS can be best interpreted from a neuroplasticity perspective, as a compensatory mechanism in response to anterior horn injury early in life. Attempted cerebral adaptation to slowly progressive LMN degeneration has been observed in other neurological conditions such as SMA [46] and lower-motor neuron predominant ALS [368, 434]. Similarly to our findings, the absence of cerebral pathology was noted in spinal-bulbar muscular atrophy (SBMA) [435] and other lower-motor neuron predominant motor neuron disease cohorts [69].

Neuroplasticity is a broad term which refers to the malleable properties of the brain to rewire and reorganise its structure, function and connections throughout life in response to experience and injury [436]. The biological processes underpinning this phenomenon are thought to be particularly effective in children, especially in the first few years of life when neurogenesis, synaptogenesis, synaptic pruning and myelination occurs physiologically [437, 438]. The efficiency of the cerebral adaptation is supported by variety of paediatric examples including early brain injury [439], brain tumour survivors [422], or surgical hemispherectomy [440, 441] in the management of intractable seizures. The biological process of neuroplasticity, although less efficient, is thought to continue throughout adolescence into adulthood [442, 443]. Structural brain plasticity has also been recognized in animal studies [444] and also observed in healthy adult populations such as professional athletes [445], highly skilled musicians [446, 447] or even in non-expert populations following targeted motor and cognitive training [448]. Successful compensatory adaptation has been also noted in adults following rehabilitation and

locomotor training after serious neurological events such as traumatic brain injury [449], spinal cord injury [450], or stroke [451]. Children with spinal cord injuries were consistently found to have better functional outcomes than those suffering spinal cord injuries in adulthood [452]. Given the young age at which our polio cohort sustained anterior horn damage due to the virus, we can speculate that considerable cortical reorganisation ensued.

Congruent to recent neurophysiological findings in adult polio patients [415, 416], we also detected changes in the motor pathways of PPS patients. These changes were evident along the entire cerebral course of the corticospinal tracts from the corona radiata through the internal capsule to the mesencephalic crura. (**Figure 3-2, 3-3**) The higher FA and lower RD in PPS compared to controls suggest well organised and well myelinated tracts [453], the opposite of what is observed in motor neuron conditions with upper motor neuron involvement [240, 242, 360, 381]. Increased brainstem density values and brainstem signal intensity was evident on whole-brain morphometry as well as region-of-interest analyses in the PPS cohort which are regions where preferential degeneration is known to occur in MND [57, 242, 244, 369]. The cerebellum has long been implicated in adaptation to primary motor system injuries and compensatory processes have been detected by structural and functional studies [369, 434]. The cerebellum plays a key role in motor learning [454], and the coordination of a variety of functions including posture, balance, speech integrating inputs from the spinal cord and cortex [455]. Many polio patients acquired considerable physical disabilities such as leg length discrepancy, muscle atrophy, foot deformations and joint misalignments after the acute infection resulting in altered gait patterns [456]. Given the longstanding physical disability experienced by polio survivors, increased cerebellar grey matter volume and superior cerebellar white matter fibre organisation may represent an adaptive process to maintain gait and posture in face of lower extremity deformities, leg length discrepancies and lower motor neuron degeneration.

This study is not without limitations. One of the drawbacks of the single-centre study design is the relatively small sample size of the PPS cohort which risks the over-interpretation of our findings and preclude subgroups analyses. We only report group-level observations in this study and don't explore approaches to categorise individual subjects into diagnostic or prognostic groups [372, 382]. The additional inclusion of a

pure UMN group, such as PLS, may have been desirable to contrast the cerebral changes observed in PPS [234]. Lastly, we acknowledge that cross-sectional imaging studies merely offer a snapshot of pathology and provide limited insights compared to longitudinal studies [457].

Notwithstanding these limitations, this neuroimaging study suggest that adult polio survivors who had their initial infection in their infancy exhibit cerebral and cerebellar adaptation and corticospinal tract reorganisation. Larger studies are needed to validate our observations in independent cohorts and verify if the cerebral compensatory processes noted herein are analogous to other lower motor neuron conditions or unique to patients who have sustained spinal cord insults in their infancy.

3.5 Conclusions

Contrary to previous reports, we found no evidence of cerebral grey or white matter degeneration in a cohort of polio survivors using a validated quantitative neuroimaging protocol. Anatomical regions which are preferentially affected in ALS, such as the corticospinal tracts and the cerebellum exhibit superior integrity in PPS than in healthy controls.

4 Extra-motor cerebral changes and clinical manifestations

4.1 Introduction

Since the introduction of the first polio vaccine in the mid-1950s, tremendous progress has been made to eradicate poliomyelitis around the globe. Thanks to the Global Polio Eradication Initiative (GPEI), two of the three wild poliovirus (WPV) serotypes have been eradicated while the transmission of the WPV type 1 remains endemic in only 2 countries across the world today [458]. While new cases of acute poliomyelitis are practically unheard of in the Western world nowadays, there are currently 15-20 million people across the globe living the lasting sequelae of the disease [2].

A large proportion of polio survivors have been reporting the development of new symptoms many years after stable functioning since recovering from the initial acute infection. This is often referred to as late-onset sequalae of poliomyelitis (LOSP) [459] or the late effects of polio (LEoP) [460]. It is estimated that between 20-85% of polio survivors satisfy the March of Dimes criteria [62, 63] for post-polio syndrome (PPS) [4, 409, 461]. Post-polio syndrome is the constellation of neurological manifestations that can affect polio patients many years after the initial poliomyelitis infection [3]. PPS typically presents as new and persistent asymmetrical muscle weakness accompanied by impaired functioning, reduced endurance, atrophy, myalgia and fasciculations [78] predominantly in the limbs but can also be observed in bulbar [79, 80] or respiratory muscles [126]. Non-motor symptoms have also consistently been reported in PPS patients. These symptoms include chronic pain, cold intolerance, fatigue [88, 459, 462], sensory manifestations [82], cognitive deficits [83, 84] as well as mood disorders [85] which all have considerable quality of life implications [89].

Generalised fatigue is one of the most commonly reported and debilitating symptoms experienced by PPS patients [462]. Fatigue is poorly understood and its aetiology is likely to be multifactorial [411]. The physical form of fatigue is the most commonly reported symptom by polio survivors [463], referring to tiredness and heaviness in the muscles, particularly after physical activity which may be associated with pain and poor sleep [459, 464-466]. Fatigue often gradually worsens throughout the day [90] and restless leg syndrome has also been linked to fatigue in PPS [98, 99]. Aside from the muscular

fatigue experienced by post-polio patients, fatigue may refer to impaired cognitive functioning [83], word findings difficulties [84], poor concentration and attention [48], memory impairment [85], and difficulty maintaining wakefulness [467]. Extra-motor manifestations are well recognised in the wider spectrum of motor neuron diseases [205], and these have been hypothetically linked to specific brain regions in polio survivors [48, 49]. Brain regions implicated in the symptomatology of post-polio syndrome include the reticular formation, posterior hypothalamus, thalamus, putamen, caudate, locus coeruleus and substantia nigra based on post-mortem studies [49-52] and signal intensity changes in the few existing neuroimaging studies [48]. Compared to other motor neuron diseases and spinal cord conditions [57, 69], there are very few imaging studies evaluating radiological changes in polio survivors and existing reports are strikingly inconsistent. For example, substantia nigra pathology was highlighted by some studies [468, 469], but not confirmed by others [59, 470]. Accordingly, the objective of this study is the comprehensive characterisation of the extra-motor features in adult polio survivors using a standardised clinical and radiological protocol.

4.2 Methods

4.2.1 Participants

Thirty-six patients with PPS, 88 patients with ALS and 117 healthy controls (HC) were included in a prospective neuroimaging study. The study was approved by the Ethics (Medical Research) Committee - Beaumont Hospital, Dublin, Ireland, and all participants provided informed consent prior to inclusion. Participating PPS patients were diagnosed according to the March of Dimes diagnostic criteria [62, 63] and ALS patients had 'probable' or 'definite' ALS according to the El Escorial criteria. [471] Inclusion criteria included the ability to tolerate the duration of MR imaging and exclusion criteria included comorbid neuro-inflammatory, neurovascular, neoplastic, psychiatric condition or prior head or spinal cord injuries. The healthy controls were unrelated to participating patients and had no known neurological or psychiatric diagnoses. Demographic and clinical details were carefully recorded for all PPS patients, including age of acute poliomyelitis, age of PPS onset, handedness, age, education and the relevant demographic variables were used as covariates in statistical models.

4.2.2 Clinical profiling

Polio survivors underwent standardised clinical assessments including the Edinburgh cognitive and Behavioural ALS Screen (ECAS) [472] and Frontal System Behavioural Scale (FrSBe) [473, 474] to examine for cognitive impairments and behavioural alterations. Age- and education based normative values validated in the Irish population were utilised to assess impairments in each domain on the ECAS [475]. Fatigue was evaluated using the Fatigue Severity Scale (FSS) [116, 476] and Piper Fatigue Scale (PFS) [477]. Based on the classification proposed by Schanke et al. [478], the patients were trichotomized into 3 groups; mildly fatigued (FSS $\leq$3.7), moderately fatigued (FSS: 3.8 – 5.8) and severely fatigued (FSS > 5.8). PFS is divided into 4 subscales measuring behavioural/severity, affective meaning, sensory and cognitive/mood attributes of fatigue where 0= no fatigue, 1-3 = mild fatigue, 4-6 = moderate fatigue and 7-10 = severe fatigue. Any psychological distress such as anxiety or depression was screened using the Hospital Anxiety and Depression Scale (HADS) [479, 480]. Modified HADS scores for use in motor neuron disease were utilised to assess for anxiety, depression and mood disorders in our PPS cohort [481].

4.2.3 Cortical thickness analyses

Cortical thickness measurements were carried out using the FreeSurfer image analysis suite. [482] Data pre-processing included the removal of non-brain tissue, segmentation of the subcortical white matter and deep grey matter structures, intensity normalization, tessellation of the grey matter-white matter boundary, and automated topology correction [483]. The labels of the Desikan-Killiany atlas [377] were used to retrieve average cortical thickness values from the superior temporal sulcus, caudal anterior cingulate gyrus, caudal middle frontal gyrus, cuneus, entorhinal cortex, fusiform gyrus, inferior parietal lobule, inferior temporal gyrus, insula, isthmus cingulate gyrus, lateral occipital sulcus, lateral orbitofrontal cortex, lingual gyrus, medial orbitofrontal cortex, middle temporal gyrus, parahippocampal gyrus, paracentral lobule, pars opercularis, pars orbitalis, pars triangularis, pericalcarine cortex, postcentral gyrus, posterior cingulate cortex, precentral gyrus, precuneus, rostral anterior cingulate cortex, rostral middle frontal gyrus, superior frontal gyrus, superior parietal lobule, superior temporal gyrus, supramarginal gyrus, frontal pole, temporal pole, and transverse temporal gyrus.

4.2.4 Subcortical grey matter analyses

Total intracranial volume (TIV) was calculated for each subject as used as a covariate in volumetric analyses. TIV estimation was performed by linearly aligning each participant's brain image to the MNI152 standard, and the inverse of the determinant of the affine registration matrix was multiplied by the size of the template. FSL-FLIRT [424] was used for registration and tissue type segmentation was performed using FSL-FAST [425, 426]. The subcortical segmentation and registration tool FIRST [484] was used to estimate the volumes of subcortical grey matter structures in the left and right hemispheres separately; the hippocampus, amygdala, thalamus, nucleus accumbens, caudate nucleus, putamen, and pallidum. Methods for subcortical grey matter segmentation and volumetry were previously described [230, 258]. FSL-FIRST uses a two-stage affine registration approach to register individual T1-weighted images to the MNI152 standard space and a model-based pipeline is subsequently implemented for the segmentation of subcortical grey matter structures using automatic boundary corrections. For brainstem segmentation, the FreeSurfer analysis suite [482] was used to pre-process T1-wieghted data including the removal of non-brain tissue, segmentation of the

subcortical structures, intensity normalization, tessellation of the grey matter-white matter boundary, and automated topology correction. A Bayesian segmentation algorithm was used for the parcellation of brainstem into the medulla oblongata, pons and midbrain, which is centred on a probabilistic atlas generated based on 49 scans [485].

4.2.5 Region of interest white matter analyses

White matter integrity metrics were retrieved from the corpus callosum (CC), cerebellar lobe, corticospinal tracts (CST), frontal lobe, occipital lobe, parietal lobe, temporal lobe, thalamus using FMRIB's FSL. Individual raw diffusion data underwent eddy current corrections and skull removal before a tensor model was fitted to generate maps of fractional anisotropy (FA). The non-linear registration and skeletonisation of each subject's image were implemented using the tract-based statistics pipeline of the FSL analysis suite. FA maps of individual participants were merged into 4D image files. Region-of-interest (ROI) FA values were retrieved from skeletonized FA maps using atlas-defined labels [486] for the corpus callosum, corticospinal tracts, frontal lobe, cerebellum, occipital lobe, parietal lobe, thalamus, and temporal lobe. Values for bilateral structures were averaged.

4.2.6 Statistics

Demographic and clinical data were interpreted using IBM Statistical Product and Service Solutions (SPSS) version 25. Group differences in subcortical volumes, regional cortical thickness, brainstem volumes were assessed using multivariate analysis of variance (MANOVA) with age, gender and education as covariates. Intergroup comparisons for subcortical volumes and brainstem volumes were also corrected for TIV. Bonferroni corrections were implemented to account for multiple comparisons. A p-value ≤ 0.05 was considered significant. Tables were generated with the estimated marginal means of volumes for each anatomical structure, standard error, between-group MANCOVA significance and p-values for Bonferroni-corrected post hoc testing. For illustration, estimated marginal means of volumes were plotted with confidence intervals to highlight group-specific volumetric traits for each structure.

4.3 Results

A total of 36 polio survivors (16 male, mean age: 66.95), 117 healthy controls (56 male, mean age: 63.38) and 88 ALS patients (56 male, mean age; 60.18) were included in this study. The mean age at acute paralytic poliomyelitis infection was 2.791 (*SD*=3.15) years and the mean interval between acute infection and PPS onset was 52.29 (*SD*=9.917) years. The majority of the recruited PPS cohort reported cold intolerance (91.7%), decreased physical endurance (80.6%), fatigue (80.6%), pain (77.8%), and poor sleep (52.8%). Polio survivors have spent a mean of 2.68 (*SD*=2.757) years in hospital during their childhood whereby a few patients spent up to 6 to 9 years in and out of hospital and underwent an average of 2 surgeries (Range 0 to 10). The average number of prescribed medication taken in PPS cohort was 3 (Range: 0 – 9) and these included statins, beta-blockers, amitriptyline, gabapentin, opiates, SSRIs which were taken in combination in a proportion of patients. Cognitive screening was performed using ECAS which was available for 29 polio patients (80.5%). Performance in each domain was interpreted using population-based age- and education-matched normative values [475]. The most commonly affected cognitive domain in the post-polio cohort was verbal fluency (65.5%), followed by language (27.6%). Memory, visuospatial and executive function deficits were less prevalent and observed in 17.2%, 17.2% and 13.8% of patients respectively. Less than 30% of patients had no deficits in any of the cognitive domains assessed, nearly half of polio patients had deficits in more than 1 domain (44.8%) and 4 patients (13.8%) had deficits in more than 2 cognitive domains. Behavioural impairment was evaluated using the FrSBe questionnaire, where the self-reported version was available for 24 patients (66.7%) and family-rating questionnaire for 20 polio survivors (55.6%). Based on FrSBe, 41.7% of patients reported apathy, 16.7% disinhibition and 25% executive dysfunction. **Table 4-1.** Similar trends were observed in the family-rating questionnaires, based on the caregiver's perspective, 55.0% noted apathy, 15% disinhibition and 30% executive dysfunction in their relative. Polio survivors were also screened for mood disorders using HADS, which was available for 34 patients (94.4%). The mean modified-HADS subscore for depression was 2.68 (*SD*=2.931), mean subscore for anxiety was 4.68 (*SD*=4.125) and mean total score was 6.91 (*SD*=6.240). The majority of polio patients did not exhibit a frank mood disorder (91.2%) based on the screening instrument, however probable depression was noted in 3 patients (8.8%), probable anxiety in 7 patients (20.6%) and probable mood disorder in only 1 patient

(2.9%). Subjective fatigue was assessed for severity using the Fatigue Severity Scale (FSS), which was available for 22 polio patients (61.1%). The mean absolute fatigue score was 41.95 (*SD*=15.063) and mean average FSS score was 4.66 (*SD*=1.688). Sixteen patients (72.7%) had a score ≥4 indicative of problematic fatigue. Based on the individual FSS scores, 13.6% were mildly fatigued, 45.5% moderately fatigued and 27.3% severely fatigued while 13.6% did not feel fatigued. The multidimensional aspect of fatigue was further assessed using the Piper Fatigue Scale (PFS), which was available for 23 polio survivors (63.9%). The mean total score for the PFS in polio patients was 3.554 (*SD*=3.0007). Based on the total PFS score, overall fatigue was predominantly mild (39.1%), however severe fatigue (21.7%) and moderate fatigue (17.4%) was also reported in some patients and the rest did not report feeling fatigued. Sensory attributes of fatigue appear to be the most severely affected (*M*=4.000, *SD*=3.0042), followed by affective (*M*=3.678, *SD*=3.6105), cognitive (*M*=3.470, *SD*=2.6647) and behavioural (*M*=3.070, *SD*=3.4478) aspects of fatigue. Severe levels of sensory fatigue were perceived in 7 (30.4%) polio survivors, of affective fatigue in 5 (21.7%), of cognitive fatigue in 2 (8.7%) and of behavioural fatigue in 4 (17.4%) ageing polio patients. **Table 4-1.**

Screening instrument and sub-domains	Scores Mean (SD)	Number of patients with impairment (% of patients assessed)
ECAS - Language	24.69 (3.475)	8 (27.6%)
ECAS - Verbal fluency	12.41 (5.742)	19 (65.5%)
ECAS - Executive	31.79 (7.889)	4 (13.8%)
ECAS - Memory	15.28 (4.511)	5 (17.2%)
ECAS - Visuospatial	10.66 (1.370)	5 (17.2%)
ECAS - Total	94.83 (16.436)	12 (41.4%)
FrSBe (self) – Apathy	61.79 (15.809)	10 (41.7%)
FrSBe (self) – Disinhibition	53.83 (10.853)	4 (16.7%)
FrSBe (self) – Executive dysfunction	56.29 (12.348)	6 (25.0%)
FrSBe (family) – Apathy	64.60 (13.705)	11 (55.0%)
FrSBe (family) – Disinhibition	55.20 (13.489)	3 (15.0%)
FrSBe (family) – Executive Dysfunction	58.70 (10.063)	6 (30.0%)
Modified HADS-D (max. 16)	2.68 (2.931)	Possible: 3 (8.8%); Probable: 3 (8.8%)
Modified HADS-A (max. 18)	4. 68 (4.125)	Possible: 2 (5.9%); Probable: 7 (20.6%)
Modified HADS-T (max. 31)	6.91 (6.240)	Possible: 2 (5.9%); Probable: 1 (2.9%)
FSS - Total score (max. 63)	41.95 (15.063)	
Average FSS score (max. 7)	4.664 (1.6876)	Mild: 3 (13.6%); Moderate: 10 (45.5%); Severe: 6 (27.3%)
PFS - Behavioural/severity (max. 10)	3.070 (3.4478)	Mild: 5 (21.7%); Moderate: 5 (21.7%); Severe: 4 (17.4%)
PFS - Affective (max. 10)	3.678 (3.6105)	Mild:3 (13.0%); Moderate: 5 (21.7%); Severe: 5 (21.7%)
PFS - Sensory (max. 10)	4.000 (3.0042)	Mild: 7 (30.4%); Moderate: 3 (13.0%); Severe: 7 (30.4%)
PFS - Cognitive (max.10)	3.470 (2.6647)	Mild: 10 (43.5%); Moderate: 6 (26.1%); Severe: 2 (8.7%)
PFS - Total score (max. 10)	3.554 (3.0007)	Mild: 9 (39.1%); Moderate: 4 (17.4%); Severe: 5 (21.7%)

Table 4- 1 The clinical profile of the post-polio syndrome cohor

4.3.1 Cortical thickness analyses

Cortical thickness analyses revealed only few areas of atrophy in the PPS cohort compared to healthy controls such as the caudal anterior cingulate gyrus, the isthmus of the cingulate gyrus, the posterior cingulate cortex and temporal pole. The inferior temporal gyrus and the medial orbitofrontal cortex also showed cortical thinning but did not reach statistical significance after Bonferroni corrections. Interestingly, increased cortical thickness was identified in the pericalcarine cortex of PPS patients compared to healthy controls. (**Table 4-2**)

Cortical Area	**Study group**	**EMM**	**Standard error**	**MANCOVA Sig. (*p*)**	**PPS vs HC**	**ALS vs HC**	**PPS vs ALS**
Banks of superior temporal sulcus	HC	2.545534	0.012909	**<.001***	1.000	**<.001***	**.016***
	PPS	2.542861	0.023836				
	ALS C9-	2.464041	0.014775				
Caudal anterior cingulate gyrus	HC	2.597427	0.017536	**.012***	**.017***	.149	.532
	PPS	2.492738	0.032382				
	ALS C9-	2.544426	0.020073				
Caudal middle frontal gyrus	HC	2.489492	0.012031	**<.001***	1.000	**<.001***	**.022***
	PPS	2.481701	0.022216				
	ALS C9-	2.410991	0.013771				
Cuneus	HC	1.853914	0.011181	**.011***	.822	**.067[t]**	**.023***
	PPS	1.880090	0.020647				
	ALS C9-	1.814566	0.012799				
Entorhinal cortex	HC	3.461052	0.026200	**.052[t]**	.632	**.052[t]**	1.000
	PPS	3.390826	0.048379				
	ALS C9-	3.365025	0.029989				
Fusiform	HC	2.712370	0.010404	**.039***	1.000	**.033***	.800
	PPS	2.696773	0.019212				
	ALS C9-	2.671526	0.011909				

Inferior parietal lobule	HC	2.400308	0.010517	**.004***	1.000	**.005***	**.063[t]**
	PPS	2.402527	0.019420				
	ALS C9-	2.349216	0.012038				
Inferior temporal gyrus	HC	2.760920	0.011537	**.019***	.335	**.020***	1.000
	PPS	2.721570	0.021303				
	ALS C9-	2.712584	0.013205				
Isthmus cingulate gyrus	HC	2.426873	0.015567	**<.001***	**.034***	**<.001***	1.000
	PPS	2.341912	0.028745				
	ALS C9-	2.322762	0.017818				
Lateral occipital sulcus	HC	2.142190	0.009896	**.044***	.471	.411	**.046***
	PPS	2.172196	0.018273				
	ALS C9-	2.119576	0.011327				
Lateral orbitofrontal cortex	HC	2.636119	0.011228	**.001***	.405	**.001***	.719
	PPS	2.600143	0.020733				
	ALS C9-	2.571312	0.012852				
Lingual gyrus	HC	1.979913	0.010261	**.014***	.657	.103	**.022***
	PPS	2.006925	0.018947				
	ALS C9-	1.946442	0.011745				
Medial orbitofrontal cortex	HC	2.404823	0.010528	**.001***	.203	**.001***	1.000
	PPS	2.363529	0.019441				
	ALS C9-	2.345860	0.012051				

Middle temporal gyrus	HC	2.832251	0.011771	**.002***	.535	**.001***	.675
	PPS	2.798307	0.021737				
	ALS C9-	2.767098	0.013474				
Parahippocampal gyrus	HC	2.807843	0.023646	.453	.920	.909	1.000
	PPS	2.756095	0.043664				
	ALS C9-	2.770465	0.027066				
Paracentral lobule	HC	2.399276	0.012696	**<.001***	1.000	**<.001***	**.022***
	PPS	2.398115	0.023444				
	ALS C9-	2.323279	0.014533				
Pars opercularis	HC	2.548378	0.012492	**.001***	1.000	**.001***	**.023***
	PPS	2.552140	0.023068				
	ALS C9-	2.478758	0.014299				
Pars orbitalis	HC	2.654233	0.015097	**.004***	1.000	**.003***	.394
	PPS	2.627315	0.027878				
	ALS C9-	2.577527	0.017281				
Pars triangularis	HC	2.433396	0.011992	**<.001***	1.000	**<.001***	**.032***
	PPS	2.416689	0.022144				
	ALS C9-	2.349362	0.013727				
Pericalcarine cortex	HC	1.579353	0.010585	**.001***	**.003***	1.000	**.001***
	PPS	1.655724	0.019546				
	ALS C9-	1.568694	0.012116				

Postcentral gyrus	HC	2.026816	0.009934	**.050[t]**	.593	.363	**.057[t]**
	PPS	2.054223	0.018343				
	ALS C9-	2.003148	0.011371				
Posterior cingulate cortex	HC	2.496416	0.013099	**.005***	**.010***	**.074[t]**	.555
	PPS	2.413093	0.024188				
	ALS C9-	2.451034	0.014994				
Precentral gyrus	HC	2.536755	0.012954	**<.001***	1.000	**<.001***	**<.001***
	PPS	2.541453	0.023920				
	ALS C9-	2.407357	0.014828				
Precuneus	HC	2.358793	0.010123	**<.001***	1.000	**<.001***	**.004***
	PPS	2.370548	0.018693				
	ALS C9-	2.298556	0.011587				
Rostral anterior cingulate cortex	HC	2.813616	0.015452	**.009***	.129	**.013***	1.000
	PPS	2.746432	0.028532				
	ALS C9-	2.745657	0.017687				
Rostral middle frontal gyrus	HC	2.318608	0.010158	**<.001***	1.000	**<.001***	**.015***
	PPS	2.305756	0.018758				
	ALS C9-	2.243121	0.011627				
Superior frontal gyrus	HC	2.645956	0.012492	**<.001***	1.000	**<.001***	**.051[t]**
	PPS	2.620431	0.023067				
	ALS C9-	2.554922	0.014299				

Superior parietal lobule	HC	2.141570	0.010957	**<.001***	.924	**.001***	**.001***
	PPS	2.165471	0.020232				
	ALS C9-	2.077999	0.012541				
Superior temporal gyrus	HC	2.799952	0.012863	**.002***	**.077[t]**	**.002***	1.000
	PPS	2.738213	0.023753				
	ALS C9-	2.732471	0.014724				
Supramarginal gyrus	HC	2.497778	0.011377	**.002***	1.000	**.002***	**.037***
	PPS	2.501160	0.021007				
	ALS C9-	2.438604	0.013022				
Frontal pole	HC	2.644002	0.016637	**<.001***	.365	**<.001***	.425
	PPS	2.588801	0.030720				
	ALS C9-	2.535369	0.019043				
Temporal pole	HC	3.799662	0.024147	**.001***	**.005***	**.007***	1.000
	PPS	3.634609	0.044588				
	ALS C9-	3.685257	0.027639				
Transverse temporal gyrus	HC	2.392149	0.017820	**.037***	1.000	.121	.081
	PPS	2.422274	0.032906				
	ALS C9-	2.335895	0.020398				
Insula	HC	2.975258	0.013481	**.018***	.279	**.020***	1.000
	PPS	2.926715	0.024893				
	ALS C9-	2.918739	0.015431				

Mean thickness	HC	2.448077	0.008256	**<.001***	1.000	**<.001***	**.009***
	PPS	2.437464	0.015245				
	ALS C9-	2.383512	0.009450				

Table 4- 2 The regional cortical thickness profile of PPS patients compared to healthy and disease controls. *highlights $p < 0.05$ [t] highlights trends at $p < 0.08$.

4.3.2 Subcortical volumetry

The systematic volumetric analysis of subcortical grey matter volumes did not reveal preferential atrophy in PPS with the exception of the left nucleus accumbens which exhibited statistically significant volume reduction in comparison to healthy controls. (**Table 4-3**). While differences did not reach statistical significance, both thalami and the left amygdala exhibited volume reductions in the PPS cohort. The segmentation of the brainstem revealed reduced medulla oblongata volumes (p=0.078) in PPS while the estimated marginal mean of the superior cerebellar peduncles were higher than in controls. To visually represent the distribution of subcortical grey matter volumes in each study group estimated marginal means were plotted for each structure with error bars representing the 95% confidence interval. **Figure 4-1**.

Subcortical structures	**Study group**	**EMM**	**Standard error**	**MANCOVA Sig. (*p*)**	**PPS vs HC**	**ALS vs HC**	**PPS vs ALS**
Thalamus (left)	HC	7575.813984	49.350562	**.010***	.164	**.013***	1.000
	PPS	7372.512694	90.895147				
	ALS C9-	7357.432805	56.420171				
Thalamus (right)	HC	7361.084223	47.091052	**.027***	.233	**.037***	1.000
	PPS	7183.181900	86.733523				
	ALS C9-	7179.355539	53.836980				
Caudate nucleus (left)	HC	3401.011185	31.464604	**.029***	.146	**.057[t]**	1.000
	PPS	3268.142522	57.952325				
	ALS C9-	3287.229438	35.971999				
Caudate nucleus (right)	HC	3576.279872	35.469151	.436	1.000	.614	1.000
	PPS	3559.973512	65.328004				
	ALS C9-	3507.227710	40.550208				
Putamen (left)	HC	4671.903297	47.810502	.355	.486	1.000	1.000
	PPS	4528.977583	88.058624				
	ALS C9-	4614.679105	54.659493				
Putamen (right)	HC	4717.015304	47.297582	.341	.436	1.000	.988
	PPS	4569.729026	87.113916				
	ALS C9-	4670.102437	54.073096				

Globus pallidus (left)	HC	1769.648332	21.350695	.578	.980	1.000	1.000
	PPS	1724.899555	39.324264				
	ALS C9-	1747.688764	24.409243				
Globus pallidus (right)	HC	1776.526489	23.010372	.956	1.000	1.000	1.000
	PPS	1772.553151	42.381101				
	ALS C9-	1765.952470	26.306674				
Hippocampus (left)	HC	3787.154422	45.669951	**.032***	1.000	**.032***	.376
	PPS	3759.952610	84.116102				
	ALS C9-	3607.460553	52.212302				
Hippocampus (right)	HC	3872.320351	44.503847	**.006***	.494	**.067[t]**	**.009***
	PPS	4004.514851	81.968341				
	ALS C9-	3715.659367	50.879150				
Amygdala (left)	HC	1212.049248	22.058099	**.016***	.195	**.020***	1.000
	PPS	1124.844575	40.627180				
	ALS C9-	1119.860322	25.217985				
Amygdala (right)	HC	1175.947792	24.693343	.138	.746	.167	1.000
	PPS	1115.088522	45.480841				
	ALS C9-	1103.261404	28.230735				
Nucleus accumbens (left)	HC	491.694184	9.669085	**.016***	**.022***	.170	.579
	PPS	435.950559	17.808772				
	ALS C9-	463.361561	11.054209				

Nucleus accumbens (right)	HC	374.305140	9.826735	**.029***	.545	**.027***	1.000
	PPS	346.250889	18.099136				
	ALS C9-	334.585064	11.234443				
Medulla	HC	4710.265507	43.758376	**.065[t]**	**.078[t]**	.471	.698
	PPS	4501.011424	80.802148				
	ALS	4615.124174	50.087268				
Pons	HC	15002.287625	152.137538	.558	1.000	.957	1.000
	PPS	14767.232363	280.929987				
	ALS	14769.644980	174.141602				
Superior cerebellar peduncle	HC	259.268639	5.348976	.313	1.000	1.000	.416
	PPS	269.313037	9.877166				
	ALS	251.989549	6.122613				
Midbrain	HC	6075.799788	46.903185	.349	.449	1.000	1.000
	PPS	5931.049276	86.609205				
	ALS	6030.118548	53.686920				

Table 4- 3 The subcortical regional profile of PPS patients compared to healthy and disease controls. *highlights $p < 0.05$ [t] highlights trends at $p < 0.08$.

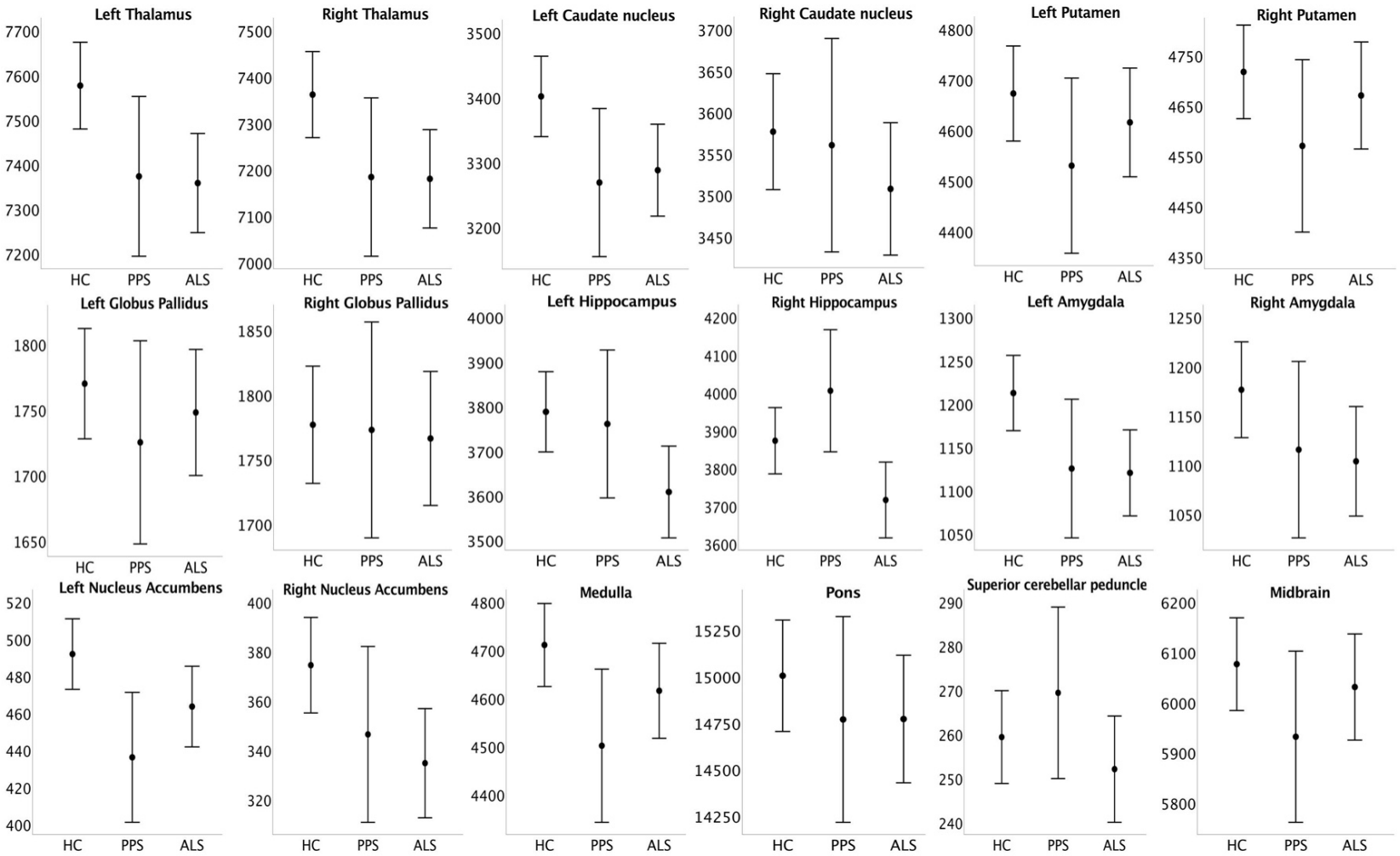

Figure 4- 1 The volumetric profile of subcortical grey matter structures in post-polio syndrome with reference to healthy controls (HC) and patients with amyotrophic lateral sclerosis (ALS). Estimated marginal means corrected for age, gender, education and total intracranial volumes are displayed. Error bars represent 95% confidence intervals.

4.3.3 Region of interest white matter metrics

The exploratory analysis of region white matter integrity did not identified FA reductions in any of the lobes. Interestingly, higher average FA was identified in the cerebellum compared to controls. **Table 4-4**. While group differences did not reach statistical significance following Bonferroni corrections, higher average FA was also noted in the frontal lobes of PPS patients compared to healthy controls. **Figure 4-2**

White matter regions	Study group	EMM	Standard error	ANCOVA Sig. (*p*)	PPS vs HC	ALS C9- vs HC	PPS vs ALS C9-
Fractional Anisotropy (FA)							
Corpus Callosum	HC	0.643937	0.003801	**<.001***	1.000	**<.001***	**.003***
	PPS	0.648745	0.007002				
	ALS C9-	0.620893	0.004343				
Cerebellar lobe (averaged)	HC	0.387673	0.002196	**.001***	**.030***	.194	**<.001***
	PPS	0.399856	0.004046				
	ALS C9-	0.381441	0.002510				
Corticospinal tract (averaged)	HC	0.523675	0.002749	**<.001***	.240	**<.001***	**<.001***
	PPS	0.533985	0.005064				
	ALS C9-	0.500254	0.003141				
Frontal lobe (averaged)	HC	0.399833	0.002348	**<.001***	.190	**<.001***	**<.001***
	PPS	0.409179	0.004326				
	ALS C9-	0.384250	0.002684				
Occipital lobe (averaged)	HC	0.371770	0.002248	**.006***	.491	**.068[t]**	**.009***
	PPS	0.378469	0.004142				
	ALS C9-	0.363887	0.002569				

Parietal lobe (averaged)	HC	0.430339	0.002472	**<.001***	.471	**.005***	**.001***
	PPS	0.437821	0.004554				
	ALS C9-	0.418238	0.002825				
Thalamus (averaged)	HC	0.332577	0.002098	**.010***	.432	.129	**.013***
	PPS	0.339137	0.003865				
	ALS C9-	0.326052	0.002397				
Temporal lobe (averaged)	HC	0.369882	0.002192	**<.001***	.150	**.022***	**<.001***
	PPS	0.379088	0.004039				
	ALS C9-	0.360802	0.002505				

Table 4- 4 The Fractional anisotropy profile of patients with post-polio syndrome compared to healthy and disease controls. *highlights $p < 0.05$ ᵗ highlights trends at $p < 0.08$.

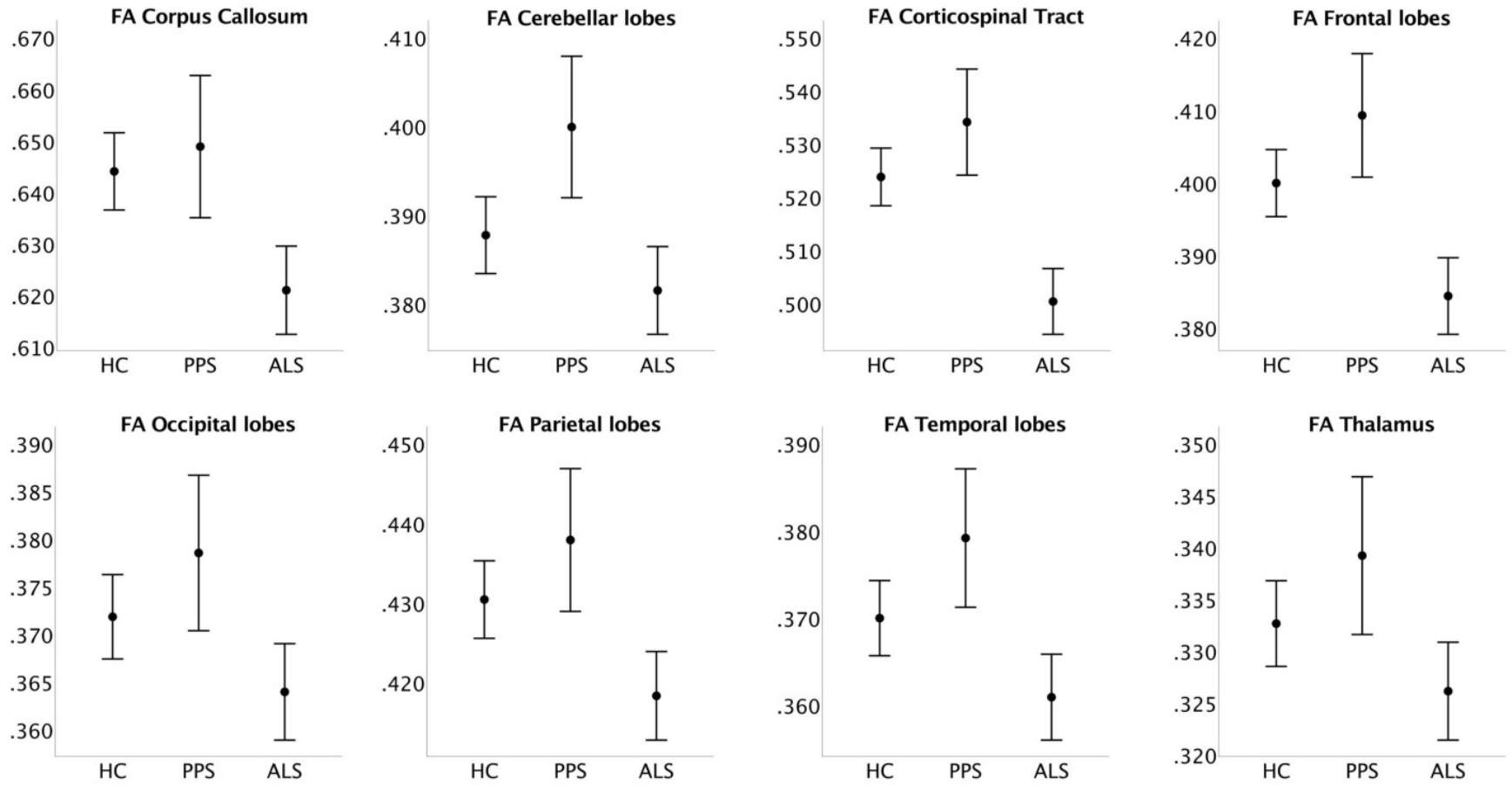

Figure 4- 2 The regional fractional anisotropy profile of post-polio syndrome with reference to healthy controls (HC) and patients with amyotrophic lateral sclerosis (ALS). Estimated marginal means corrected for age, gender, education and total intracranial volumes are displayed. Error bars represent 95% confidence intervals.

4.4 Discussion

The overarching objective of this study was the systematic characterisation of extra-motor manifestations in post-polio syndrome and the evaluation of the anatomical underpinnings of these symptoms. While neuropsychological deficits, fatigue, sensory disturbances are often reported in post-polio syndrome, there is a paucity of studies using a standardised battery of screening tests and a standardised quantitative neuroimaging protocol. We have detected a high incidence of extra-motor manifestations with relatively limited cerebral neurodegenerative change.

Our standardised screening instruments captured a high prevalence of verbal fluency deficits, language deficits, apathy and considerable fatigue in this patient cohort. In line with previous reports we also detected deficits in executive functioning, memory and visuospatial skills [48, 83, 84, 487]. The ascertainment of these deficits is important for the support of these patients, optimisation of their rehabilitation strategy, personalisation of their exercise regimes, and improvement of their quality of life. The majority of studies in post-polio syndrome focus on mobility, physical disability, motor function and exercise tolerance overlooking the myriad of extra-motor manifestations. The systematic evaluation of these symptoms is hugely important as they not only have major quality of life implications for polio survivors but may contribute to caregiver burden also. While fatigue is consistently reported by patients, other deficits such as apathy may be under-recognised, despite its likely impact on adherence to rehabilitation efforts, participation in clinical trials and clinic attendances.

Radiologically, we have only identified relatively limited cortical atrophy, subcortical degeneration and changes in white matter integrity. Cortical atrophy was limited to the cingulate gyrus, and the temporal pole. Similarly to the cortical analyses, only limited subcortical atrophy was detected in the PPS cohort affecting the left nucleus accumbens. Brainstem changes in PPS were also moderate with only a trend of atrophy detected in the medulla oblongata (p=0.078). No FA reductions were identified in this study to indicate white matter degeneration in any of the lobes. Our findings indicate an apparent discordance between the severity of extra-motor manifestations and the relative lack of cerebral radiological abnormalities. While nucleus accumbens volume reductions and cingulate gyrus atrophy may contribute to the higher incidence of apathy in the PPS

cohort, no widespread frontotemporal or subcortical change was captured in this neuroimaging study to account for the spectrum of extra-motor clinical manifestations. Cortical thinning of temporal pole is also likely to contribute to the cognitive deficits observed in our cohort. The temporal pole mediates a multitude of language functions, not only related to comprehension but also to language production [488]. Temporal pole thinning has been observed in other neurological conditions, notably language variants of frontotemporal lobar degeneration [206]. Cortical thinning in the anterior cingulate gyrus has been linked to verbal fluency deficits in multiple sclerosis [489]. Posterior cingulate gyrus injury has been associated with has been associated with a number of manifestations such as attention deficits [490, 491], behavioural or language deficits [492]. Brain lesions acquired at the time of acute poliomyelitis have been hypothesised to occur in almost all cases parallel to the acute anterior horn cell injury [49, 493]. It was theorised that polio-induced cerebral lesions may be associated with the late-onset fatigue and attention deficits observed in polio survivors many years later [48, 49]. In contrast to previous post-mortem [49-51] and neuroimaging studies [48], we did not detect extensive subcortical pathological changes within the thalamus, putamen, or caudate.

The majority of our patients reported self-perceived fatigue which was considered problematic (FSS≥4). While fatigue is one of the most commonly reported symptoms by polio survivors [462], it refers to a multitude of experiences such as 'lack of energy', reduced physical endurance, physical discomfort, lack of motivation and sleepiness [494]. The physical aspects of fatigue are well-recognised in post-polio syndrome [88, 463], but cognitive attributes such as word findings difficulties, poor concentration, limited attention span, and difficulty in maintaining wakefulness are poorly characterised and seldom evaluated systematically [48, 83-85, 487, 495]. The severity of subjective fatigue reported in our polio survivors group was slightly lower than that found in previous reports [88, 116, 459, 496, 497]. Fatigue is a cardinal symptom of a range of neurological conditions such as multiple sclerosis [498], Parkinson's disease [499], traumatic brain injury [500], stroke [501] and amyotrophic lateral sclerosis [502]. The anatomical correlates of fatigue have been repeatedly evaluated, but no consensus findings can be identified by integrating the observations of existing studies. In multiple sclerosis, fatigue has been linked to a multitude of structures including the thalamus, pallidum, superior cerebellar peduncles, temporal cortex as well as fronto-parietal white matter [503-506]. In Parkinson's disease, fatigue has been associated with the atrophy of the caudate and

putamen [507]. In our opinion, fatigue in PPS is likely to be multi-factorial, owning to poor sleep, polypharmacy, sub-clinical or overt respiratory compromise, low mood, rather than a sequela of focal cerebral change or underlying neuroendocrine pathology.

The implications of contracting acute paralytic poliomyelitis at a young age may have had a long lasting effect on the lives of many individuals - physically but also psychologically, one of which is how they fit into the world around them. Many of the polio survivors spent prolonged periods of time in isolation in hospitals, separated from their families with limited visits, which was accommodated through a window. Their schooling was frequently interrupted due to medical issues and numerous surgeries related to the lasting effects of the polio. As a result, many survivors of polio would fall behind in attaining certain milestones in their education in contrast to their peers and as snowball effect, have a knock on effect on their self-esteem and self-confidence, distance themselves from their peers and feel isolated. However, on the other hand, many of the polio survivors is also observed to be high-achievers as a way to compensate for what they may have lost in their physical abilities. This is also seen in other lower motor neuron disease such as spinal muscular atrophy (SMA) [508]. Additionally, the stigma attached with living with a physical disability, feeling feared, judged and pitied may also impact their ability to socialise thus leading to social anxiety and isolation. These are all important factors which could be influencing the apathetic behaviour and anxious behaviour detected in our cohort.

Our study also highlights the utility of standardised screening instruments which are adapted for motor disability. ECAS has been originally developed to capture cognitive changes in patients with ALS, but as it is relatively brief and has been adapted to patients with poor mobility, bulbar impairment and motor disability, it is a particularly useful tool for other motor neuron disease cohorts, such as patients with PPS. Validated fatigue questionnaires are just as useful, as different patients refer to different aspects of fatigue and these instruments allow the nuanced characterisation of the specific facets of fatigue. Unless blatantly obvious, subtle behavioural impairment is difficult to detect in a routine clinical consultations, therefore structured questionnaires such as FrSBe are useful to interrogate domains and the incorporate the caregiver's perspective in the assessments. While disinhibition is relatively easy to recognise during a cursory clinical assessment, apathy may remain under-recognised or mistaken for low mood or depression.

This study is not without limitations. The relatively small sample size precludes the estimation of the prevalence of cognitive deficits in PPS and the reliance of screening instruments only allows the identification of impaired domains instead of in-depth neuropsychological characterisation. The cross-sectional design of the study merely offers of snapshot of extra-motor clinical and radiological changes instead of evaluating progressive processes and describing the trajectory of cognitive changes in this cohort. Larger studies are needed to systematically characterise the extra-motor profile of PPS with the interrogation of additional neuropsychological domains such as social cognition. The additional assessment of respiratory function, polysomnography, and endocrine parameters may help to elucidate the underpinnings of fatigue in more detail. Notwithstanding these limitations, our study demonstrates the relative integrity of the frontotemporal cortex and subcortical structures in a cohort of PPS patients.

4.5 Conclusions

Post-polio syndrome is associated with a range of extra-motor features, such as fatigue, language deficits and apathy, which may be relatively under recognised unless specifically assessed. The recognition of these impairments is important for individualised rehabilitation and screening for these imprisonments should be integrated in the multidisciplinary care of these patients. The absence of widespread cerebral change gives cause for optimism for neurocognitive interventions and cognitive rehabilitation. Fatigue is likely to be multifactorial in PPS and the meticulous assessment of the likely causes may help to improve the quality of life of PPS patients.

5 Cerebellar changes in poliomyelitis survivors

5.1 Introduction

Poliomyelitis reached epidemic proportions in the early 20th century and is still endemic today in some parts of Asia [408]. It predominantly affected young children under the age of five, induced asymmetrical flaccid paralysis in less than 1% and carried a mortality rate of 5-10% owing to respiratory failure and vasomotor complications [407]. Today, there is an estimated 15-20 million polio survivors across the globe who are living with the lasting effects of the original disease [2], of which 20-85% meet the March of Dimes criteria for post-polio syndrome (PPS) [461]. The 'late effects of polio' (LEoP) is a broad term that refers to new symptoms due to motor-unit dysfunction or secondary to the biomechanical alterations from polio-related surgeries, musculoskeletal deformities and long-term compensatory adaptations to residual muscle weakness and atrophy [509]. The term post-polio syndrome (PPS) denotes persistent and progressive motor decline, but other symptoms such as generalised fatigue, chronic pain, cold intolerance, sleep disturbances, neuropsychological deficits and sensory impairments have also been reported in the literature [2, 411].

Despite poliomyelitis primarily affecting the anterior horns of the spinal cord, supraspinal involvement, including cerebellar pathology, has been described in the literature. Previous post-mortem studies revealed polio-induced lesions affecting the subcortical regions such as substantia reticularis [49, 52, 493], putamen, caudate, locus coeruleus, substantia nigra [49], vestibular nuclei [50, 493], hypothalamus and thalamus [49, 52, 510, 511]. Reports of cortical involvement are inconsistent; it is thought to be limited to motor and pre-motor areas [49] and the cerebellar pathology limited to the vermis and deep cerebellar nuclei. Accounts of involvement of cerebellar cortical layers are conflicting [49, 493, 512, 513]. Sporadic cerebellar manifestations such as ataxia, nystagmus, vertigo and intention tremor had been linked to poliovirus type 1 [514-516]. The rarity of frank cerebellar findings was hypothesised to be due to the mildness of cerebellar degeneration and the challenge of ascertaining cerebellar signs in the presence of widespread lower motor neuron degeneration [513]. In more recent studies, however, functional [415, 416, 517] and structural [518] supraspinal changes are thought to represent adaptive processes in response to longstanding lower motor neuron loss.

The cerebellum is implicated in a multitude of motor and cognitive functions spanning from motor control, coordination, maintenance of balance, posture, motor learning, to higher cognitive and affective regulatory processes [519]. Each cerebellar hemisphere can be subdivided into 3 lobes and further delineated into 10 smaller lobules (I-X); anterior lobe (lobules I-V), posterior lobe (lobules VI-IX) and flocculonodular lobe (lobule X) [520]. The grey matter of the cerebellar cortex is arranged into three layers (from outer to inner; molecular, Purkinje and granular) which contains both excitatory and inhibitory neurons important in integrating cerebellar inputs and modulating cerebellar outputs. Deeply embedded within the cerebellar white matter are the deep cerebellar nuclei (dentate, emboliform, globose and fastigial) which are the sole output structures of the cerebellum. Climbing fibres, originating from the contralateral inferior olivary nucleus, and mossy fibres, largely via the pontocerebellar tract, are the two main input routes to the cerebellar cortex and provide excitatory signals to Purkinje fibres. Functionally, the cerebellum has been suggested to be topographically organised. The 'motor cerebellum' is thought to primarily include the anterior lobe and lobule VIII of posterior lobe. Lobules VI and VII constitute the 'cognitive cerebellum' and posterior vermis is generally regarded as the 'limbic cerebellum' [520, 521].

The majority of quantitative imaging studies in motor neuron diseases focus either on amyotrophic lateral sclerosis [423, 522] or on primary lateral sclerosis [380, 397]. Cerebral studies of lower motor neuron predominant conditions and in poliomyelitis survivors are scarce. With the striking paucity of neuroimaging studies and conflicting accounts of cerebellar involvement in poliomyelitis survivors, our primary objective is the comprehensive, multiparametric characterisation of cerebellar involvement *in vivo* in adult poliomyelitis survivors. An additional aim of our study is the evaluation of cerebro-cerebellar connectivity via the targeted assessment of cerebellar peduncles. Based on recent studies of long-term poliomyelitis survivors, we hypothesize that focal cerebellar changes may be detected confined to lobules that mediate sensorimotor processes instead of global cerebellar degeneration.

5.2 Methods

5.2.1 Ethics statement

The study was approved by the institutional ethics committee (Beaumont Hospital, Dublin Ireland) and all participants provided written informed consent.

5.2.2 Participants

Forty-three adult poliomyelitis survivors and 100 healthy controls (HC) enrolled in a prospective cross-sectional neuroimaging study. All participating adult poliomyelitis survivors had a verified diagnosis of poliomyelitis in infancy/childhood supported by clinical and electromyographic findings. Inclusion criteria included the ability to tolerate the duration of MR imaging and exclusion criteria included comorbid neuro-inflammatory, neurovascular, neoplastic, psychiatric conditions or prior head or spinal cord injuries. Healthy controls were unrelated to participating patients and had no known neurological diagnoses or previous head injury. Demographic and clinical details were carefully recorded for polio survivors including age at acute poliomyelitis infection. Functional disability was appraised using the revised ALS rating scale (ALSFRS-r) [332].

5.2.3 Cortical thickness and volume analyses

A validated segmentation algorithm [21] was implemented to estimate cerebellar cortical thickness and lobular volumes. Pre-processing steps included the ‘denoising’ of raw structural data in native space, inhomogeneity corrections, affine registration to the Montreal Neurological Institute (MNI) space, inhomogeneity corrections in MNI space, cerebellar cropping, low dimensional non-linear registration estimation, and intensity normalization. Cerebellar volume metrics were generated for each lobule using a patch-based segmentation algorithm [22] separately in the right and left cerebellar hemispheres. As a quality control step, the accuracy of tissue-type segmentation and anatomical parcellation were individually reviewed for each subject. Cortical volume and thickness values were retrieved from lobules I-II, III, IV, V, VI, VIIB, VIIIA, VIIIB, IX, X, Crus I and Crus II.

5.2.4 Morphometry

Voxelwise grey matter changes were explored using region-of–interest morphometry in FMRIB's FSL suite. First, total intracranial volumes (TIV) were calculated for each participant, which was subsequently used as a covariate in the morphometric analyses and for the interpretation of lobular volumes. TIV calculations have been previously described [232, 242], briefly, participant's brain image was aligned to the MNI152 standard, and the inverse of the determinant of the affine registration matrix was calculated and multiplied by the size of the template. For spatial registration, FMRIB's FSL-FLIRT and for tissue type segmentation, FSL-FAST was utilised. Output partial grey matter, white matter and CSF volumes were added to determine TIV. Raw T1w data underwent skull-removal (BET), motion-corrections and tissue-type segmentation. The accuracy of skull removal and segmentation was visually inspected in each subject for quality control. Individual grey-matter partial volume images were aligned to the MNI152 standard space using affine registration. Permutation-based non-parametric inference was for the voxelwise analyses implementing the threshold-free cluster enhancement (TFCE) method. Design matrices included group membership, age, sex and TIV. Voxelwise statistics were run in the cerebellar mask derived from 'label 1' of the MNI structural atlas [523]. Output statistical maps were thresholded at $p < 0.05$ FWE TFCE and the Diedrichsen probabilistic atlas was used as underlay to help the localisation of statistically significant clusters.

5.2.5 Voxelwise white matter analyses

Subsequent to eddy current corrections and skull removal; a tensor model was then fitted to the raw diffusion data in FSL to generate maps of axial diffusivity (AD), fractional anisotropy (FA), mean diffusivity (MD) and radial diffusivity (RD). For non-linear registration and skeletonisation of individual images, FMRIB's software library's tract-based statistics module was utilised. For the two-way, voxelwise comparison of diffusivity parameters between poliomyelitis survivors and healthy controls permutation-based non-parametric inference was used restricting the analyses the cerebellar portion of the study-specific white matter skeleton. The design matrix used for permutation included group-membership, and age and sex as covariates. The threshold-free cluster enhancement (TFCE) method was applied and results considered significant at a $p < 0.01$ TFCE family-wise error (FWE).

5.2.6 Cerebellar peduncle assessments

The labels (1,11,12,13,14) of the JHU-ICBM atlas was utilised to generate masks for the left and right inferior, the middle and the left and right superior cerebellar peduncles Average FA, AD and RD values were retrieved from the merged skeletonised diffusion data using these masks from each subject for group comparisons.

5.3 Results

The demographic profile of healthy controls and polio survivors are summarised in **Table 5-1.** While the healthy controls were age- and gender- matched, they were also included as covariates in our analyses.

	Healthy Controls (n= 100)	**Poliomyelitis survivors (n=43)**	***p* value**
Age (years)	66.13 (6.472)	66.14 (6.707)	.994
Gender (male)	52 (52%)	18 (41.9%)	.266
Age at acute poliomyelitis (years)	-	2.60 (2.991)	-
ALSFRS-r total (max. 48)	-	39.65 (7.368)	-

Table 5- 1 The demographic profile of study participants

5.3.1 Cortical thickness analyses

Compared to healthy controls, polio survivors exhibited increased cortical thickness in lobules I-II, and lobule III in the right hemisphere, and in lobules VIIIA and VIIIB bilaterally controlling for age and sex. **Table 5-2**. A trend in increased cortical thickness was also detected in lobules I-II and III in the left hemisphere. No frank cortical thinning was identified, but a trend of cortical thinning was noted in the left Crus I and right Crus II in poliomyelitis survivors.

Cerebellar region	**Study group**	**EMM**	**Standard error**	**ANCOVA Sig. (*p*)**
Lobules I-II (right)	HC	1.345115	0.031503	**.038***
	Polio	1.465976	0.048126	
Lobules I-II (left)	HC	1.396892	0.034921	**.067[t]**
	Polio	1.514657	0.053347	
Lobule III (right)	HC	3.065033	0.031523	**.010***
	Polio	3.215933	0.048156	
Lobule III (left)	HC	3.187530	0.034797	**.065[t]**
	Polio	3.305935	0.053157	
Lobule IV (right)	HC	4.755922	0.017989	.126
	Polio	4.806548	0.027480	
Lobule IV (left)	HC	4.904537	0.014732	.709
	Polio	4.894459	0.022506	
Lobule V (right)	HC	4.737028	0.018026	.915
	Polio	4.733494	0.027538	
Lobule V (left)	HC	4.898671	0.015243	.891
	Polio	4.894833	0.023287	
Lobule VI (right)	HC	4.924036	0.011482	.389
	Polio	4.905869	0.017540	
Lobule VI (left)	HC	4.974966	0.011298	.319
	Polio	4.954307	0.017259	

Crus I (right)	HC	4.640872	0.024112	.307
	Polio	4.595695	0.036835	
Crus I (left)	HC	4.581111	0.021624	**.076[t]**
	Polio	4.510507	0.033034	
Crus II (right)	HC	4.571318	0.025911	**.077[t]**
	Polio	4.486973	0.039582	
Crus II (left)	HC	4.375609	0.026474	.853
	Polio	4.366600	0.040442	
Lobule VIIB (right)	HC	4.786736	0.017746	.143
	Polio	4.738892	0.027109	
Lobule VIIB (left)	HC	4.612632	0.021329	.518
	Polio	4.637905	0.032584	
Lobule VIIIA (right)	HC	4.635282	0.016534	**.046***
	Polio	4.696278	0.025258	
Lobule VIIIA (left)	HC	4.654906	0.017625	**.003***
	Polio	4.753642	0.026924	
Lobule VIIIB (right)	HC	4.557164	0.026441	**.001***
	Polio	4.726895	0.040393	
Lobule VIIIB (left)	HC	4.513289	0.033450	**.003***
	Polio	4.696155	0.051100	
Lobule IX (right)	HC	3.771612	0.039352	.225
	Polio	3.859370	0.060116	
Lobule IX (left)	HC	3.588834	0.043782	.866
	Polio	3.575345	0.066883	
Lobule X (right)	HC	2.244308	0.040101	.938
	Polio	2.238572	0.061261	
Lobule X (left)	HC	2.479459	0.046601	.402
	Polio	2.551171	0.071189	

Table 5- 2 Cerebellar cortical thickness in poliomyelitis survivors and healthy controls. Statistical comparisons are corrected for age and gender. *highlights $p < 0.05$ [t] highlights trends at $p < 0.08$.

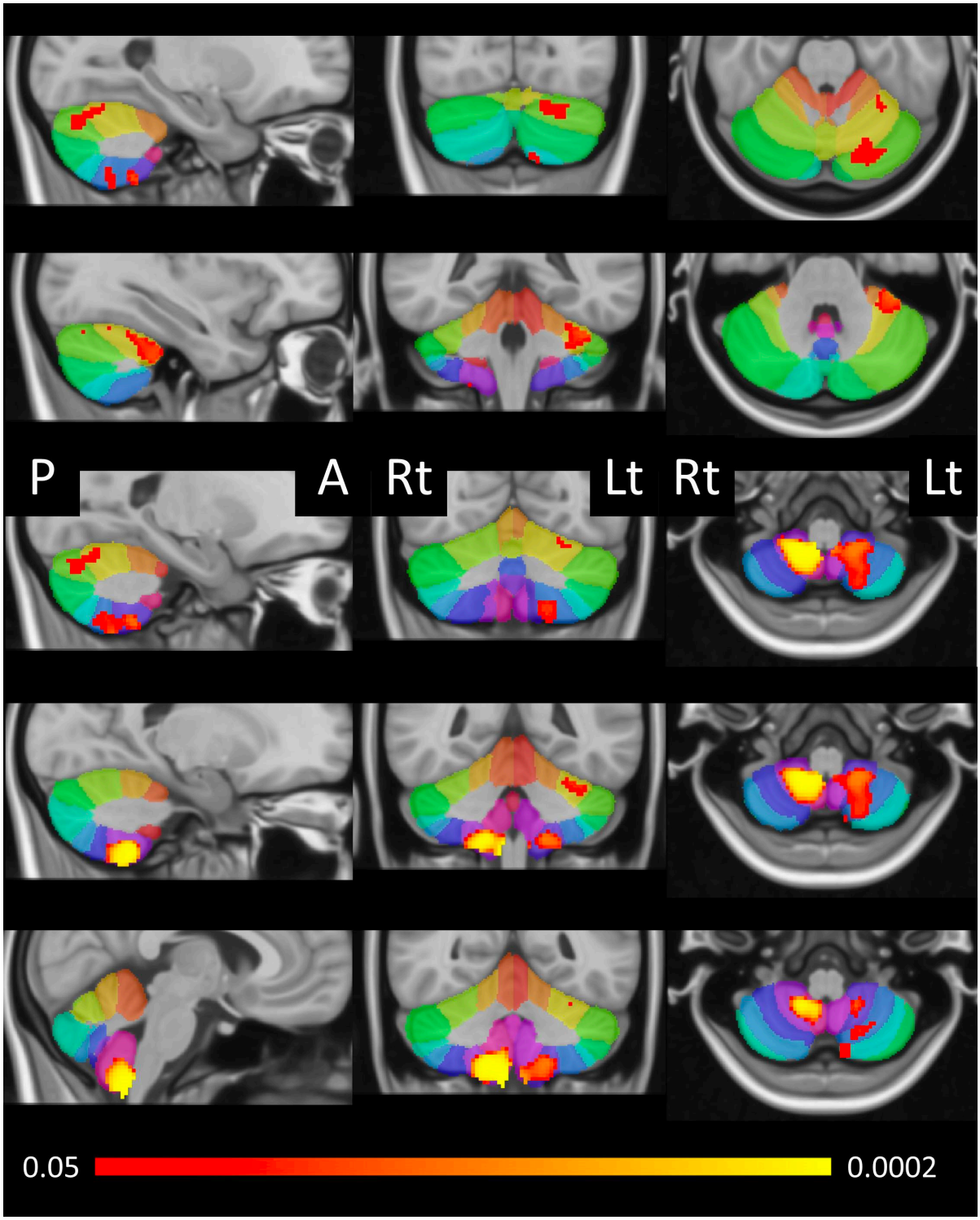

Figure 5- 1 Morphometric patterns of increased cerebellar GM partial volumes in poliomyelitis survivors indicated in red - yellow at p <0.05 FWE TFCE corrected for age, gender and total intracranial volumes (TIV)

5.3.2 Grey matter volumes

Increased grey matter volume was detected in the left lobule VIIIB of poliomyelitis survivors. A trend for increased grey matter volume was also observed in lobule X of the left cerebellar hemisphere. **Table 5-3**.

Cerebellar region	**Study group**	**EMM**	**Standard error**	**ANCOVA Sig. (*p*)**
Lobules I-II (right)	HC	0.034213	0.001009	.474
	Polio	0.035546	0.001547	
Lobules I-II (left)	HC	0.029124	0.001016	.457
	Polio	0.027728	0.001558	
Lobule III (right)	HC	0.488477	0.009796	.612
	Polio	0.497645	0.015020	
Lobule III (left)	HC	0.499655	0.010724	.912
	Polio	0.497474	0.016443	
Lobule IV (right)	HC	1.928698	0.031254	.550
	Polio	1.963218	0.047923	
Lobule IV (left)	HC	2.078443	0.032415	.416
	Polio	2.029758	0.049704	
Lobule V (right)	HC	3.279021	0.045692	.852
	Polio	3.263248	0.070061	
Lobule V (left)	HC	3.564292	0.044253	.518
	Polio	3.511443	0.067855	
Lobule VI (right)	HC	7.870917	0.107912	.890
	Polio	7.843433	7.516257	
Lobule VI (left)	HC	7.861064	0.106252	.531
	Polio	7.983838	0.162921	
Crus I (right)	HC	11.118685	0.159449	.531
	Polio	10.934478	0.244491	
Crus I (left)	HC	11.127808	0.165490	.609
	Polio	10.971721	0.253753	

Crus II (right)	HC	7.018721	0.105569	.344
	Polio	6.834148	0.161873	
Crus II (left)	HC	6.842425	0.099249	.628
	Polio	6.753806	0.152183	
Lobule VIIB (right)	HC	4.179157	0.058421	.132
	Polio	4.016144	0.089580	
Lobule VIIB (left)	HC	3.983546	0.060471	.793
	Polio	3.954225	0.092722	
Lobule VIIIA (right)	HC	4.792981	0.066827	.743
	Polio	4.752624	0.102469	
Lobule VIIIA (left)	HC	4.921682	0.068260	.581
	Polio	4.991243	0.104667	
Lobule VIIIB (right)	HC	3.409387	0.059077	.088
	Polio	3.596341	0.090585	
Lobule VIIIB (left)	HC	3.330394	0.051710	**.016***
	Polio	3.563492	0.079289	
Lobule IX (right)	HC	2.858097	0.042857	.688
	Polio	2.889833	0.065714	
Lobule IX (left)	HC	2.698948	0.041592	.818
	Polio	2.681282	0.063775	
Lobule X (right)	HC	0.574727	0.007499	.519
	Polio	0.583663	0.011498	
Lobule X (left)	HC	0.575363	0.007213	**.060^{t}**
	Polio	0.600540	0.011060	

Table 5- 3 Cerebellar cortical volumes in poliomyelitis survivors and healthy controls. Statistical comparisons are corrected for age, sex and intracranial volumes. *highlights $p < 0.05$ ^{t} highlights trends at $p < 0.08$.

5.3.3 Cerebellar peduncles analyses

Diffusivity alterations in the cerebellar peduncles are presented in **Table 5-4**.

Region	Study group	EMM	Standard error	ANCOVA Sig. (*p*)
Fractional Anisotropy (FA)				
Superior peduncle (left)	HC	0.625407	0.004077	.651
	Polio	0.621869	0.006394	
Superior peduncle (right)	HC	0.619614	0.004211	.316
	Polio	0.611502	0.006603	
Middle peduncle	HC	0.520931	0.003512	**.001***
	Polio	0.543068	0.005507	
Inferior peduncle (left)	HC	0.516051	0.004326	**.045***
	Polio	0.499289	0.006784	
Inferior peduncle (right)	HC	0.512402	0.004462	**.061[t]**
	Polio	0.496299	0.006997	
Axial Diffusivity (AD)				
Superior peduncle (left)	HC	0.001380	0.000009	**<.001***
	Polio	0.001453	0.000014	
Superior peduncle (right)	HC	0.001398	0.000009	**<.001***
	Polio	0.001465	0.000014	
Middle peduncle	HC	0.001046	0.000005	.774
	Polio	0.001043	0.000008	
Inferior peduncle (left)	HC	0.001081	0.000007	**.002***
	Polio	0.001122	0.000011	
Inferior peduncle (right)	HC	0.001092	0.000007	.097
	Polio	0.001114	0.000011	
Radial Diffusivity (RD)				
Superior peduncle (left)	HC	0.000447	0.000006	**.016***
	Polio	0.000474	0.000009	
Superior peduncle (right)	HC	0.000464	0.000006	**.015***
	Polio	0.000492	0.000009	

Middle peduncle	HC	0.000433	0.000004	**.002***
	Polio	0.000410	0.000006	
Inferior peduncle (left)	HC	0.000460	0.000005	**<.001***
	Polio	0.000501	0.000007	
Inferior peduncle (right)	HC	0.000464	0.000005	**.001***
	Polio	0.000495	0.000007	
Mean Diffusivity (MD)				
Superior peduncle (left)	HC	0.000758	0.000006	**<.001***
	Polio	0.000800	0.000009	
Superior peduncle (right)	HC	0.000775	0.000006	**<.001***
	Polio	0.000817	0.000009	
Middle peduncle	HC	0.000638	0.000004	**.031***
	Polio	0.000621	0.000006	
Inferior peduncle (left)	HC	0.000667	0.000005	**<.001***
	Polio	0.000708	0.000007	
Inferior peduncle (right)	HC	0.000673	0.000005	**.002***
	Polio	0.000701	0.000007	

Table 5- 4 Diffusivity alterations in cerebellar peduncles correcting for age and sex. *highlights $p < 0.05$ [t] highlights trends at $p < 0.08$.

Higher fractional anisotropy was detected in middle cerebellar peduncle and lower FA in the left inferior peduncle. In the superior cerebellar peduncles, higher AD, RD and MD were identified.

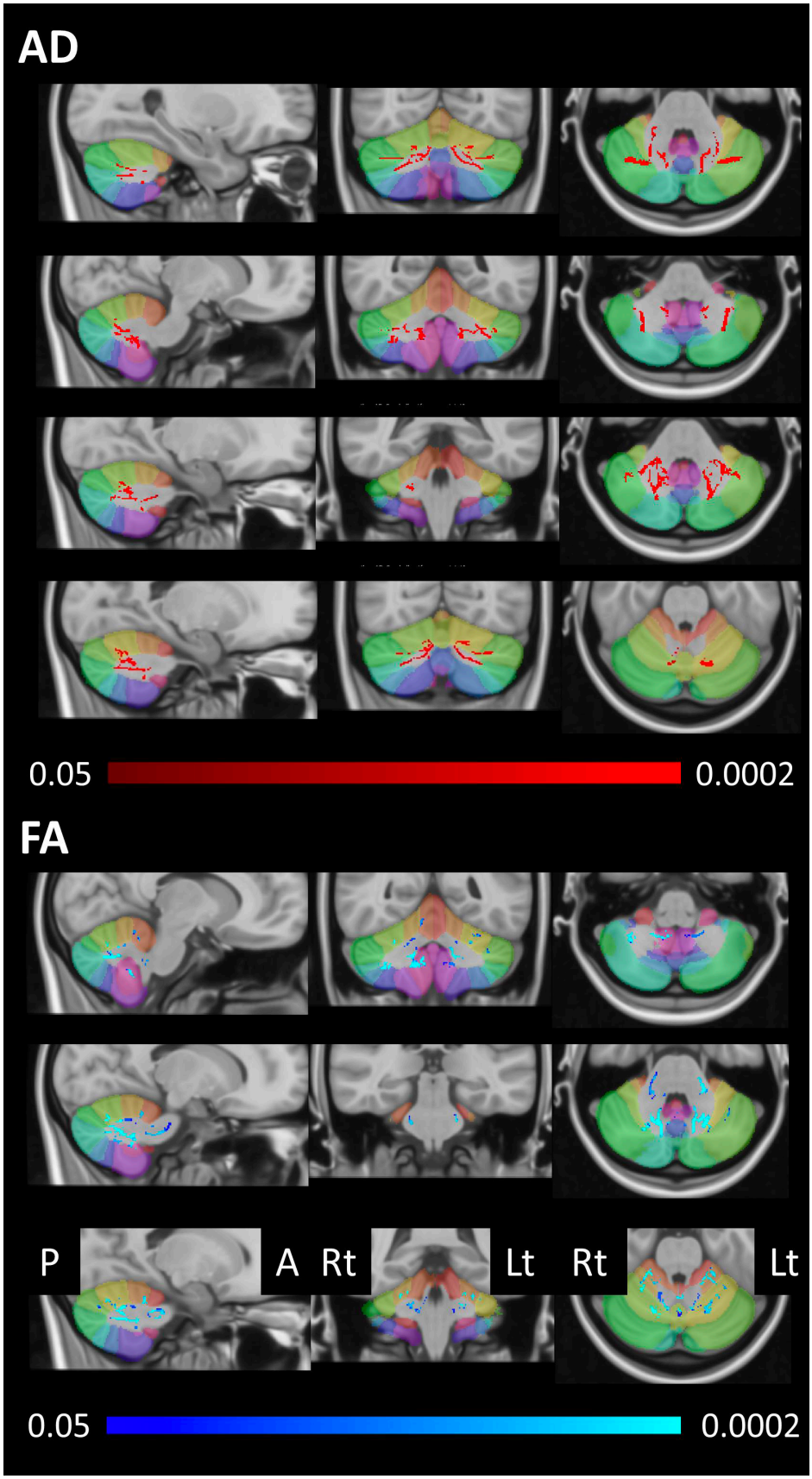

Figure 5- 2 Voxelwise patterns of increased fractional anisotropy (FA) and reduced axial diffusivity (AD) in poliomyelitis survivors as identified by tract-based spatial statistics at $p < 0.05$ TFCE adjusted for age and gender.

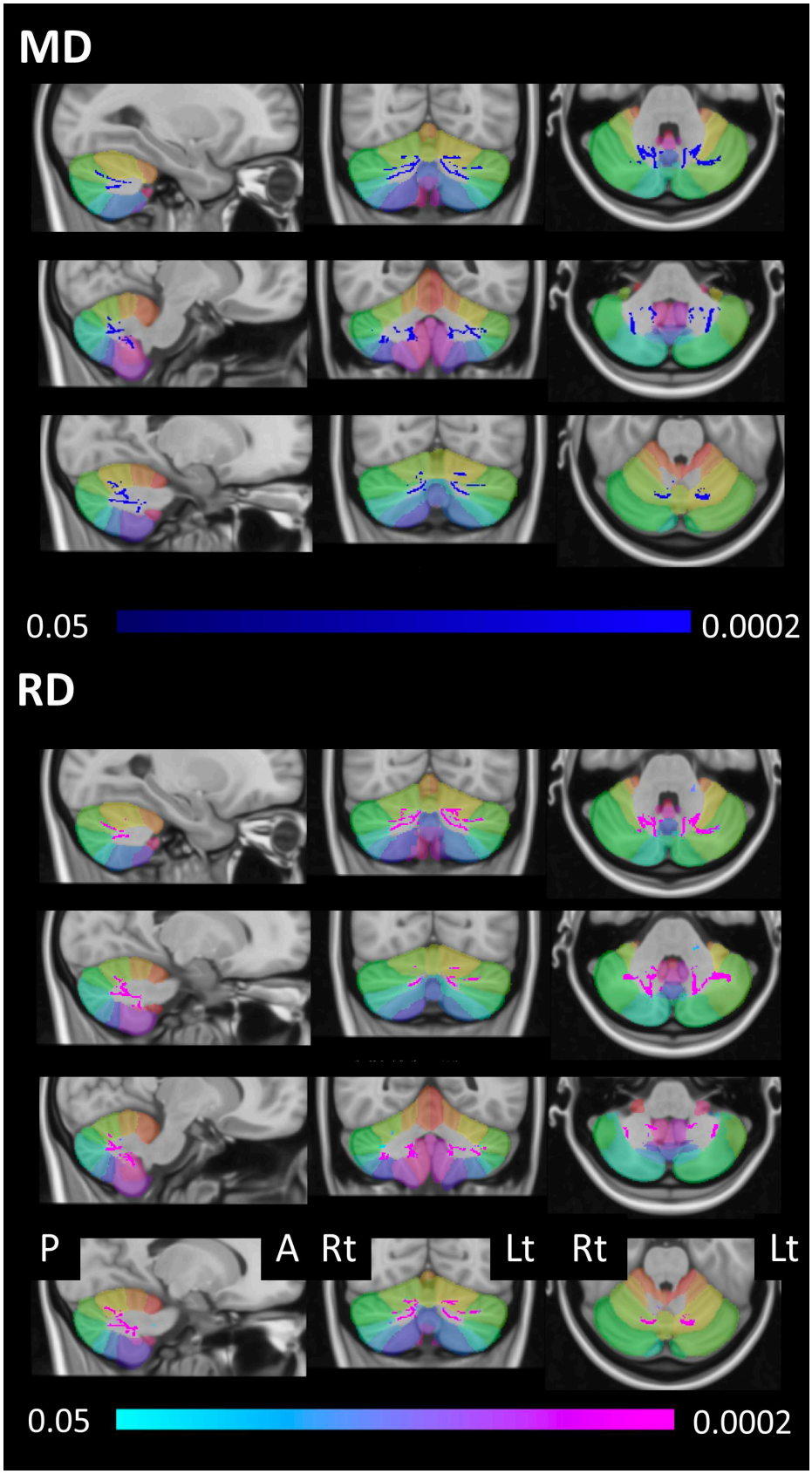

Figure 5- 3 Voxelwise patterns of reduced mean diffusivity (MD) and reduced radial diffusivity (RD) in poliomyelitis survivors as identified by tract-based spatial statistics at $p < 0.05$ TFCE adjusted for age and gender.

5.3.4 Voxel-wise analyses

No foci of cerebellar atrophy were identified in poliomyelitis survivors. Voxel-wise grey matter analyses revealed increased grey matter partial volumes in lobules VIIIb and IX bilaterally, and in lobules VI, VIIIa and crus I in the left cerebellar hemisphere in poliomyelitis survivors in contrast to healthy controls adjusting for age, sex and TIV (**Figure 5-1**). On voxel-wise analyses of white matter microstructure, higher FA and lower AD, RD and MD were detected in lobules VI and IX and crus I bilaterally in adult poliomyelitis survivors with reference to healthy controls (**Figure 5-2 and Figure 5-3**). Polio survivors also exhibited higher FA in lobule V and lower AD, RD and MD in crus II bilaterally compared to healthy individuals. Lastly, compared to healthy controls, higher FA with lower AD was detected bilaterally in the middle cerebellar peduncles of adult polio survivors.

5.4 Discussion

Our study provides compelling in vivo evidence of cerebellar changes in adult poliomyelitis survivors with reference to healthy controls. In contrast to previous post-mortem studies [49, 51, 512, 513] and sporadic clinical reports [514-516], the cohort of adult poliomyelitis survivors evaluated in this study did not exhibit cerebellar atrophy. On the contrary, polio survivors exhibited hypertrophic changes in the cerebellum. The changes were focal and relatively symmetrical affecting selective cerebellar lobules as opposed to the entire cerebellum. Increased cortical thickness was detected in lobules I-III, VIIIa and VIIIb and increased grey matter volume was found in lobules VIIIb. In addition to the higher cerebellar cortical thickness, our data also showcased increased white matter organisation in the cerebellar peduncles, in particular in the middle and inferior cerebellar peduncles. Anatomical patterns of increased grey matter metrics were relatively concordant across retrieved cortical thickness, cortical volume and morphometric analyses and the three analyses have unanimously demonstrated the lack of atrophic changes. The interpretation of increased grey matter in an adult disease-cohort requires the careful review of the literature as the vast majority of imaging studies report disease-associated patterns of atrophy [234, 256, 381]. It is conceivable that a reporting bias exists for neurodegenerative changes and also that one-way contrasts may sometimes be run assuming that a given patient cohort exhibit atrophy with respect of

demographically matched healthy controls. Increased grey matter metrics, such as volumes, partial volumes, thickness etc. are often explored from an adaptive remodelling perspective, especially if the identified anatomical patterns of hypertrophy are functionally congruent with function-specific cortical areas [46, 434]. Adaptive cerebral reorganisation due to longstanding insult or slowly progressive neurodegenerative change is often described based on functional MRI observations but relatively rarely captured on structural imaging [46, 254, 524, 525].

Neuroplasticity refers to the unique ability for the nervous system to modify and reorganise itself both structurally and functionally in a dynamic manner in response to injury [436]. This process is well-recognised to be more efficient in children [526], especially during the first few years of life, when neurogenesis, synaptogenesis, synaptic pruning and myelin formation and remodelling is heightened [437]. This is supported by superior functional outcomes following CNS insult in young children compared to adults and has been demonstrated by functional recovery of language centres [527, 528], visual systems [529, 530] and sensorimotor networks [422, 439, 531]. Neuroplasticity is a particularly well-recognised trait of the cerebellum which plays a crucial role in motor learning [532], acquisition and adaptation of movement to environmental demands. The term 'cerebellar reserve' has been coined for the structure's unparalleled ability to adapt to lesions and bored broadly to supratentorial pathology [533]. In animal studies, motor training has led to glial hypertrophy, thickening of the molecular layer, synaptogenesis, and increased dendrite numbers in stellate cells [534-536]. Efficient cerebellar adaptation has been described in young patients with traumatic brain injury [537] and other forms of cerebellar degeneration [538]. Unique cerebellar plasticity has also been demonstrated in healthy populations such as musicians [539, 540], athletes [541, 542] and other performers [543] following motor training.

The detected patterns of increased cerebellar metrics suggest function specific foci. Lobules I-III of anterior lobe, and lobules VI, VIIIa and VIIIb of posterior lobe primarily mediate sensorimotor processes [520, 544, 545]. The somatotopic architecture of the cerebellum has been extensively investigated [521, 546]. Paradigm-based functional neuroimaging studies suggest that lobules II-III represent the legs and toes with respect to both tactile stimulation and during motor tasks [547, 548]. Injury of these lobules is thought to result in gait and posture ataxia or lower limb ataxia (lobules I-III, VIII and

IX) [549-551]. These are the same lobules which exhibited hypertrophic alterations in our cohort of poliomyelitis survivors. Our voxelwise analyses also indicate increased lobule VI and lobule IX volumes. Lobule IX is considered essential for visually guided movement [552], while lobules VI has been implicated in motor learning [553]. Poliomyelitis survivors are predominantly affected in their lower extremities due to residual polio-induced musculoskeletal deformities such as leg-length discrepancy, asymmetrical muscle weakness and joint arthrodesis for which they have learned to compensate for with altered gait patterns, posture and balance [456] all of which are adaptive processes mediated by cerebellar motor learning.

Our diffusivity findings also indicate enhanced cerebellar peduncles integrity in poliomyelitis survivors decades after their infection particularly in the middle (MCP) and inferior cerebellar peduncles (ICP). The superior cerebellar peduncles (SCP) are the main cerebellar output projections from the deep cerebellar nuclei to the contralateral cerebral cortex via the thalamus, known as cerebello-thalamo-cortical (CTC) projections. The MCP constitute entirely of afferent fibres from the contralateral cerebral cortex via the pontine nuclei, known as the cortico-ponto-cerebellar (CPC) tract. These two tracts form a closed feed-forward and feed-back loop that enables the cerebellum to modulate and coordinate both motor and non-motor processes [554, 555]. Anatomically, the ICP consists of both cerebellar efferent and afferent fibres integrating information from the vestibular nuclei, mediating eye movements and head positioning. The ICP also encompasses afferent spinal fibres carrying proprioceptive and cutaneous information from the limbs to the cerebellum, which are crucial for posture, locomotion and muscle control [556]. Higher FA and lower RD in middle cerebellar peduncles may be interpreted as well organised fibre and suggests well myelinated cortico-ponto-cerebellar tracts. MCP alterations have been described in musicians [557] and are thought to represent an adaptive change to repetitive sensorimotor and cognitive demands. The progressive recruitment of the cerebellum to carry out motor tasks has been observed in several neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) [369, 434, 524], Parkinson’s disease (PD) [558] or Huntington’s disease (HD) [559]. However, it is more commonly detected as increased activation or increased metabolism on functional imaging, and only rarely appreciated on structural imaging [560].

The majority of studies in neurodegenerative conditions focus on the description of atrophy patterns [392] and connectivity changes [252, 356] underpinning specific clinical phenotypes [57, 406]. The targeted characterisation of compensatory changes is seldom specifically pursued and adaptive structural alterations in particular are rarely evaluated [561]. Our observations may have implications for other cohorts with acute anterior horn injury, such as patients with spinal cord infarction or spinal cord injuries [69]. Depending on the anatomical extent and age at the primary insult, adaptive processes may take place to improve functional outcomes, which provides a very strong rationale for meticulous multidisciplinary interventions such as individualised physiotherapy and occupational therapy. Our findings may also be of relevance for non-traumatic, non-vascular, lower motor neuron conditions such as progressive muscular atrophy (PMA), spinal muscular atrophy (SMA), Spinal-Bulbar Muscular Atrophy (SBMA) or patients with LMN-predominant ALS [393, 562-564]. Depending on the rate of progression of the underlying neurodegenerative process, attempted compensatory processes may be at play to mitigate or slow down the functional decline. This further highlights that rehabilitation efforts are also very important in progressive neurodegenerative conditions, and not just in neurovascular conditions and post trauma [391]. The presence of compensatory mechanisms may also explain the apparent divergence between disease burden and the functional profile in some motor neuron disease phenotypes [395]. Compensatory processes may also delay symptom manifestation despite considerable presymptomatic disease [296, 384, 386]. Beyond the academic interest of describing adaptive processes in vivo, the demonstration of marked cerebellar neuroplasticity may support the rationale for non-invasive plasticity-inducing electrostimulation as part of multidisciplinary rehabilitation strategies [565-567].

This study is not without limitations. Our study has a cross-sectional design and included a relatively small sample of poliomyelitis survivors which did not allow for further stratification for age at initial infection or disability severity. We have only evaluated cerebral changes in this study even though quantitative spinal protocols are increasingly available [58, 396] and combined cord-brain studies are likely to elucidate proposed compensatory processes further. We also acknowledge the inherent inclusion bias to patients with less severe disability; patients with severe polio-induced deformities were less likely to attend our research facility or tolerate the duration of the MRI protocol. Furthermore, our observations are mere snapshots of cerebellar alterations many decades

after the original illness. The longitudinal trajectory of putative compensatory processes could not be explicitly demonstrated due to the cross-sectional design of the study. Notwithstanding these limitations, our findings suggest radiological evidence of possible cerebral reorganisation decades after severe cord injury in infancy. We hypothesise that similar compensatory processes may occur in other spinal conditions and that the younger the age at initial injury, the more successful these processes may be. While these hypotheses remain to be confirmed in purpose-designed longitudinal protocols, our findings highlight the importance of specifically evaluating hypertrophic changes in any neuroimaging studies instead of running a single contrast to characterise patterns of atrophy.

5.5 Conclusions

Considerable cerebellar reorganisation occurs decades after poliomyelitis infection which may indicate compensation to anterior horn insult in infancy. Similar processes may take place in other anterior horn conditions and in particular in motor neuron diseases. Instead of focusing on neurodegenerative changes primarily, academic neuroimaging studies should seek to evaluate evidence for putative adaptive changes as well.

6 Evaluation of subcortical structures in poliomyelitis survivors

6.1 Introduction

Despite the poliovirus being 99% globally eradicated today, there are approximately 15-20 million people across the world who are currently living with the lasting sequelae of the initial infection [1]. Of those, it is estimated that 20-85% of polio survivors meet the March of Dimes criteria [63] for post-polio syndrome (PPS). PPS refers to a non-contagious condition, characterised by slowly progressive neurological decline after a prolonged period of stable neurological functioning following the initial infectious event, usually 15 – 40 years. Commonly reported symptoms include new persistent and progressive muscle weakness, decreased muscular endurance, asymmetrical muscle atrophy, decreased ambulation, pain in muscles and joints and fasciculations predominantly within the limbs but can also affect the respiratory and bulbar musculature [77-80]. While motor dysfunction is the hallmark of the disease, PPS patients also reported non-motor symptoms such as generalised fatigue, intolerance to cold temperatures, chronic pain [568], sleep disorders and sleep-related breathing disorders [97, 569], sensory deficits [82] as well as cognitive [83, 84] and behavioural impairments [86], all of which have substantial impact on the patient's well-being and quality of life [89, 570].

Although poliomyelitis has traditionally been regarded as a spinal cord condition, evidence from the past literature suggest otherwise. Previous post-mortem studies have highlighted polio-induced lesions extending beyond the spinal cord to affect more central brain regions such as reticular formation of pons and medulla, putamen, caudate, locus coeruleus, substantia nigra, vestibular nuclei, hypothalamus, thalamus, motor cortex and cerebellum [49-52, 513, 571]. These widespread polio-induced insults to central nervous system has been hypothesised to be responsible for the generalised fatigue and cognitive deficits experienced by polio survivors later in life [487]. These findings were further supported by some neuroimaging studies [48, 468, 469] but not corroborated by other more recent studies [59, 470]. On the contrary, recent neurophysiological [415, 416] and

neuroimaging studies [518, 572] have demonstrated supraspinal alterations interpreted as compensatory reorganisation of the cerebral cortex.

The thalamus is a paired structure predominantly made of grey matter located in the dorsal part of the diencephalon. It is composed of approximately 60 distinct nuclei, each of which have unique inputs and output projections, mostly to the cerebral cortex. Its many connections include those to the hippocampus, mammillary bodies and fornix via the mammillothalamic tract, to the cerebral cortex via thalamocortical radiations and the spinothalamic tract originating in the spinal cord that carry sensory information about pain, temperature, crude touch and pressure. The thalamus is recognised to play an important role in numerous functions including relaying and processing sensory and motor signals prior to interpretation by higher cortical areas [573], regulation of sleep and wakefulness [574, 575], encoding of different components of pain [576], executive functioning such as planning, cognitive control, working memory and decision making [577-579], processing language [580] as well as the regulation of stress, mood and motivation due to its links to the limbic system [581]. The amygdala is a key structure of the limbic system which mediates many aspects of emotion and memory. It is an almond-shaped structure that is located deep in the temporal lobe, adjacent to the hippocampus and medial to the hypothalamus. The amygdala is made up of approximately 13 nuclei that can be organised into 5 major groups namely the basolateral nuclei, cortical-like nuclei, central nuclei, other amygdaloid nuclei and the extended amygdala with extensive connections with several cortical and subcortical structures. It is well known in the regulation of emotions such as fear, anxiety and aggression, emotional learning, social cognition, reward-based decision-making and its modulating effect on the encoding and consolidation of memory [582-586].

Despite post-mortem reports of widespread central involvement and extra-motor clinical observations indicating cerebral pathology, very few studies have been dedicated to the exploration of cerebral involvement in PPS and the in-depth characterisation of considered affected structures *in vivo.* Accordingly, our study objective was the exploration and systematic evaluation of subcortical brain structures, in particular the thalamus and the amygdala in patients with PPS, which have not been undertaken previously. While we did not identify any evidence of thalamic or amygdala atrophy in our previous neuroimaging study, we hypothesise that structural changes may occur

within specific thalamic and amygdalar nuclei as opposed to global changes affecting the entire structure. A secondary objective of this chapter is to explore whether central compensatory changes detected in polio survivors also extend to subcortical areas, notably the thalamus and amygdala.

6.2 Methods

6.2.1 Ethics statement

This study has been approved by institutional Ethics Medical Research Committee (Beaumont Hospital, Dublin, Ireland) and all participants provided informed written consent prior to inclusion.

6.2.2 Participants

A total of 241 participants comprising of 36 patients with post-polio syndrome (PPS), 88 patients with amyotrophic lateral sclerosis without *C9orf72* gene mutation (ALS C9-) and 117 healthy controls were recruited between September 2018 and April 2022 and enrolled in this prospective, single-centre neuroimaging study. The participating PPS patients were diagnosed as per the March of Dimes diagnostic criteria [62, 63] and the ALS participants had 'probable' or 'definite' ALS according to the El Escorial criteria [471]. Inclusion criteria for the study included the ability to tolerate the duration of the MR imaging protocol while the exclusion criteria included co-morbid neuro-inflammatory, neurovascular, neoplastic, psychiatric condition and prior head or spinal cord injuries. Healthy controls were unrelated to the participating patients and had no known neurological diseases or previous head or spinal cord injuries. Demographics details were carefully recorded including age, gender, handedness and education for all participants in addition to age at initial poliomyelitis infection and age of onset of PPS in polio patients.

6.2.3 TIV and total brainstem volumes

In each subject, total intracranial volume (TIV) was calculated to be included as a covariate in relevant statistical models. TIV estimations were performed using the FMRIB Software Library (FSL). Following brain extraction with BET, [587] brain images were linearly aligned to the MNI152 standard image, the inverse of the determinant of the affine registration matrix was calculated and multiplied by the size of

the template. FSL-FLIRT was used for registration to template, [424] and tissue type segmentation was performed using FSL-FAST. [425]

6.2.4 Thalamic segmentation

As described previously [423], a Bayesian inference was used to segment the thalamus into 25 sub-regions, using a probabilistic atlas developed based on histological data [588] (**Figure 6-1**). The thalamus was parcellated into the following nuclei in each hemisphere: antero-ventral (AV), latero-dorsal (LD), lateral posterior (LP), ventral anterior (VA), ventral anterior magnocellular (VAmc), ventral lateral anterior (VLa), ventral lateral posterior (VLp), ventral posterolateral (VPL), ventromedial (VM), central medial (CeM), central lateral (CL), paracentral (Pc), centromedian (CM), parafascicular (Pf), paratenial (Pt), reuniens/medial ventral (MV-re), mediodorsal medial magnocellular (MDm), mediodorsal lateral parvocellular (MDl), lateral geniculate (LGN), medial geniculate (MGN), limitans/suprageniculate (L-SG), pulvinar anterior (PuA), pulvinar medial (PuM), pulvinar lateral (PuL), and pulvinar inferior (PuI). The raw volume estimates of the above nuclei were averaged between left and right and merged into the following 10 core group of nuclei defined based on their distinctive physiological function: “Anteroventral”, “Lateral geniculate”, “Medial geniculate”, “Pulvinar-limitans” (PuA, PuM, PuL, PuI, L-SG), “Laterodorsal”, “Lateroposterior”, “Mediodorsal-paratenial-reuniens” (MDm, MDl, MV-re, Pt), “Motor hub” (VA, VAmc, VLa, VLp), “Sensory hub” (VPL, VM), “Intralaminar” (CeM, CL, Pc, CM, Pf). “Total thalamic volume” was defined as the mean of the left and right thalamus volume estimates.

6.2.5 Amygdala segmentation

A Bayesian inference was used to parcellate the amygdala into 9 sub-regions using a probabilistic atlas developed based on histological data in the FreeSurfer analysis suite [588, 589] (**Figure 6-2**). The amygdala was parcellated into the following nuclei in each hemisphere: lateral nucleus (LN), basal nucleus (BN), accessory basal nucleus (ABN), anterior amygdaloid area (AAA), central nucleus (CeN), medial nucleus (MN), cortical nucleus (CN), cortico-amygdaloid transition (CAT), and paralaminar nucleus (PN). The raw volume estimates of the above nuclei were averaged between left and right. “Total amygdala volume” was defined as the mean of the left and right total amygdala volume estimates.

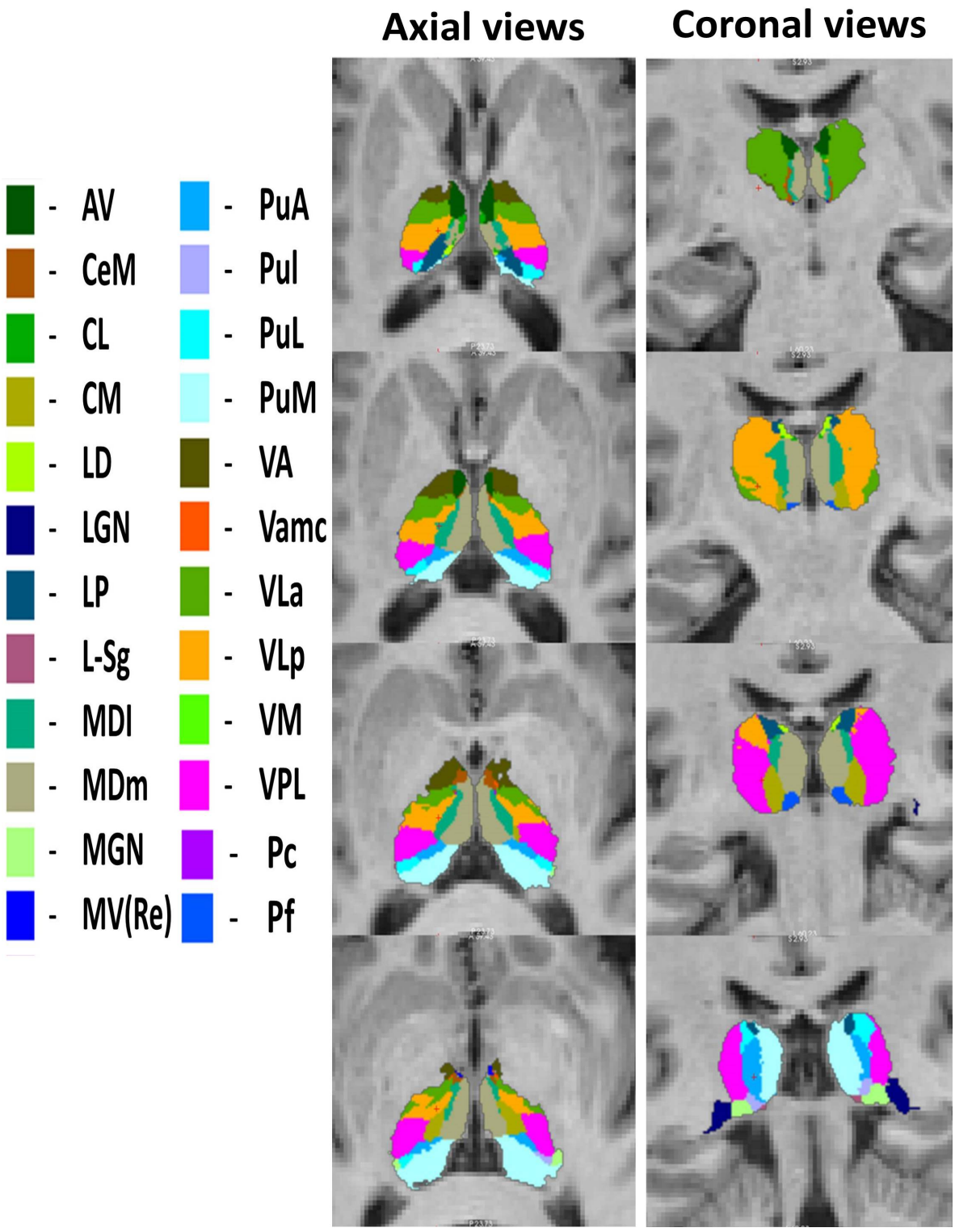

Figure 6- 1 Thalamic segmentation on representative axial and coronal views**.**

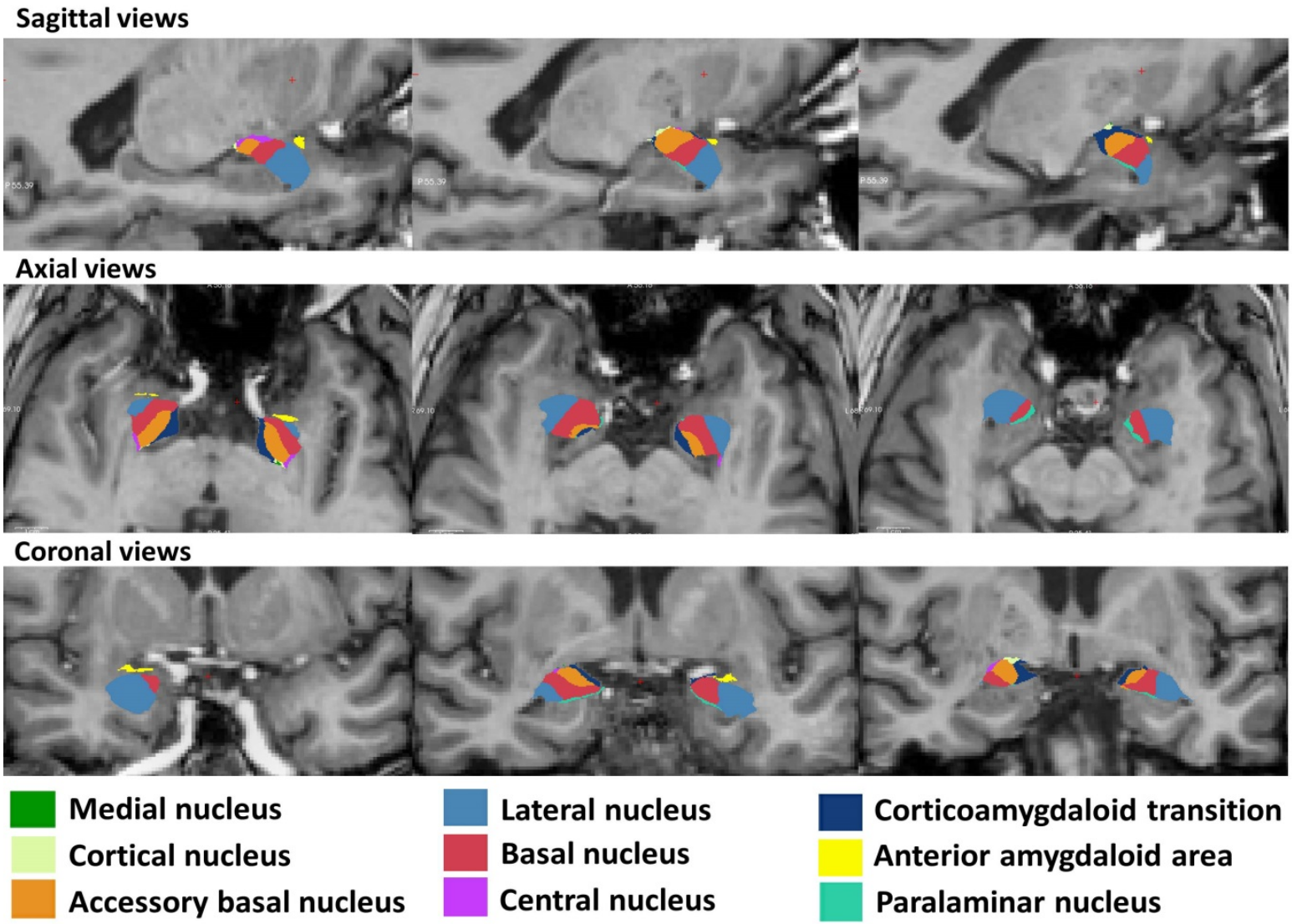

Figure 6- 2 Amygdala parcellation on representative views.

6.2.6 Statistics

Demographics data was statistically interpreted using IBM Statistical Product and Service Solutions (SPSS) version 25. Group differences in thalamic nuclei volumes and amygdala nuclei volumes were assessed using multivariate analysis of variance (MANOVA) with age, gender and education as covariates. Intergroup comparisons for subcortical volumes were also corrected for total intracranial volumes (TIV). Bonferroni corrections were implemented to account for multiple comparisons. A p-value ≤ 0.05 was considered significant. Tables were generated with the estimated marginal means of volumes for each anatomical structure, standard error, between-group MANCOVA significance and p-values for Bonferroni-corrected post hoc testing. For illustration, estimated marginal means of volumes were plotted with confidence intervals to highlight group-specific volumetric traits for each structure. Additionally, radar plots were utilised to highlight comparative profiles of the study groups in reference to healthy individuals.

6.3 Results

The demographic profile of the healthy controls, PPS and ALS participants is detailed in **Table 6-1**. Our imaging analyses were also adjusted for age, gender, education and TIV as covariates.

	HC ***n*=117**	**PPS** ***n*=36**	**ALS C9-** ***n*=88**	***p* value**
Age, years (SD)	63.38 (11.914)	66.97 (5.629)	60.18 (10.311)	.052
Gender, male (%)	56 (47.9%)	16 (44.4%)	56 (63.6%)	.059
Education, years (SD)	13.31 (3.294)	12.56 (3.691)	13.58 (3.169)	.064
Handedness, right (%)	109 (93.2%)	34 (94.4%)	81 (92%)	.855
Age at initial poliomyelitis infection, months (SD)	N/A	33.50 (37.867)	N/A	-
Age at PPS onset, years (SD)	N/A	55.08 (9.075)	N/A	-

Table 6- 1 The demographic profile of the participating HC, PPS and ALS C9-.

6.3.1 Thalamic nuclei volumes

Our analyses demonstrated statistically significant intergroup volume differences in the ventromedial (VM), central medial (CeM), central lateral (CL), mediodorsal medial magnocellular (MDm) and mediodorsal lateral parvocellular (MDl) nuclei while the volume of the thalamus as a whole structure was comparable between the 3 groups (p= .222). Our PPS cohort showed a lack of atrophy in all the thalamic nuclei when compared to healthy controls. On the other hand, the ALS group displayed significant atrophic changes within the ventromedial (VM) and mediodorsal medial magnocellular (MDm) nuclei, and a trend within the mediodorsal lateral parvocellular (MDl) nuclei in reference to healthy controls. Furthermore, the ALS group showed significant atrophy in the central lateral (CL) nucleus in contrast to the PPS group (p=.029). There was also a trend in smaller volumes of the limitans/suprageniculate (L-SG) nuclei denoted in our PPS group when compared to the ALS group (p=.054). **(Table 6-2)**

Thalamic nuclei	**Study group**	**EMM**	**Standard error**	**ANCOVA Sig. (*p*)**	**PPS vs HC**	**ALS C9- vs HC**	**PPS vs ALS C9-**
Anteroventral (AV)	HC	119.666481	1.616862	**.066[t]**	1.000	.110	.240
	PPS	120.661735	2.985621				
	ALS C9-	114.465955	1.850713				
Latero-dorsal (LD)	HC	22.980281	0.677146	.205	1.000	.641	.296
	PPS	24.133640	1.250387				
	ALS C9-	21.687076	0.775084				
Lateral posterior (LP)	HC	111.608194	1.515199	.452	1.000	.674	1.000
	PPS	111.327692	2.797895				
	ALS C9-	108.783497	1.734346				
Ventral anterior (VA)	HC	365.501218	3.852771	.593	1.000	.921	1.000
	PPS	362.749604	7.114345				
	ALS C9-	359.458567	4.410008				
Ventral anterior magnocellular (VAmc)	HC	28.698792	0.340588	.520	1.000	.942	1.000
	PPS	28.829653	0.628914				
	ALS C9-	28.172612	0.389848				
Ventral lateral anterior (VLa)	HC	609.397256	6.304523	.342	1.000	.505	1.000
	PPS	596.937156	11.641634				
	ALS C9-	596.052227	7.216363				

Ventral lateral posterior (VLp)	HC	804.737349	8.414333	.285	1.000	.382	1.000
	PPS	788.377134	15.537510				
	ALS C9-	785.027777	9.631321				
Ventral posterolateral (VPL)	HC	842.094863	9.455391	.118	.534	.163	1.000
	PPS	814.824073	17.459878				
	ALS C9-	814.071938	10.822950				
Ventromedial (VM)	HC	20.449817	0.261238	**.037***	.484	**.038***	1.000
	PPS	19.666023	0.482389				
	ALS C9-	19.445771	0.299021				
Central medial (CeM)	HC	54.426141	0.843203	**.039***	1.000	.112	.095
	PPS	55.696224	1.557018				
	ALS C9-	51.724078	0.965157				
Central lateral (CL)	HC	31.310843	0.599433	**.022***	.559	.189	**.029***
	PPS	33.007884	1.106884				
	ALS C9-	29.596263	0.686131				
Paracentral (Pc)	HC	3.131053	0.043979	.162	1.000	.318	.362
	PPS	3.170985	0.081210				
	ALS C9-	3.021767	0.050340				
Centromedian (CM)	HC	253.038682	2.893731	.293	.450	.871	1.000
	PPS	244.117250	5.343427				
	ALS C9-	248.343038	3.312259				

Parafascicular (Pf)	HC	62.371921	0.758856	.727	1.000	1.000	1.000
	PPS	61.266172	1.401267				
	ALS C9-	61.653290	0.868611				
Paratenial (Pt)	HC	6.393679	0.067319	.293	.668	.552	1.000
	PPS	6.217840	0.124308				
	ALS C9-	6.256277	0.077056				
Reuniens/medial ventral (MV-re)	HC	8.434790	0.185360	.098	1.000	.197	.247
	PPS	8.614274	0.342277				
	ALS C9-	7.909622	0.212169				
Mediodorsal medial magnocellular (MDm)	HC	672.221164	7.264278	**.028***	1.000	**.026***	.458
	PPS	665.473942	13.413873				
	ALS C9-	642.759201	8.314930				
Mediodorsal lateral parvocellular (MDl)	HC	257.626119	2.590203	**.049***	1.000	.056[t]	.368
	PPS	256.969807	4.782947				
	ALS C9-	248.229153	2.964831				
Lateral geniculate (LGN)	HC	164.094978	1.922915	.397	.538	1.000	1.000
	PPS	158.564826	3.550763				
	ALS C9-	162.233904	2.201031				
Medial geniculate (MGN)	HC	108.110975	1.279264	.096	1.000	.177	.266
	PPS	109.156469	2.362228				
	ALS C9-	104.393179	1.464287				

Limitans/suprageniculate (L-SG)	HC	20.719084	0.515099	**.059[t]**	.298	.837	**.054[t]**
	PPS	18.898283	0.951157				
	ALS C9-	21.575335	0.589599				
Pulvinar anterior (PuA)	HC	205.746992	1.714853	.888	1.000	1.000	1.000
	PPS	204.309017	3.166567				
	ALS C9-	204.686170	1.962877				
Pulvinar medial (PuM)	HC	926.064244	7.953927	.952	1.000	1.000	1.000
	PPS	929.877003	14.687345				
	ALS C9-	924.427993	9.104325				
Pulvinar lateral (PuL)	HC	173.939815	2.348122	.724	1.000	1.000	1.000
	PPS	174.020904	4.335931				
	ALS C9-	176.688569	2.687737				
Pulvinar inferior (PuI)	HC	171.393088	1.984749	.818	1.000	1.000	1.000
	PPS	169.420607	3.664944				
	ALS C9-	172.162056	2.271809				
Whole thalamus	HC	6044.157819	49.529446	.222	1.000	.254	1.000
	PPS	5966.288194	91.458734				
	ALS C9-	5912.825316	56.693024				

Table 6- 2 Anatomical thalamic nuclei volumes of healthy controls (HC), patients with post-polio syndrome (PPS) and *C9orf72* negative ALS (ALS C9-). Statistical tests corrected for multiple comparisons (Bonferroni), age, sex, education and TIV. *highlights $p < 0.05$ [t] highlights trends at $p < 0.08$.

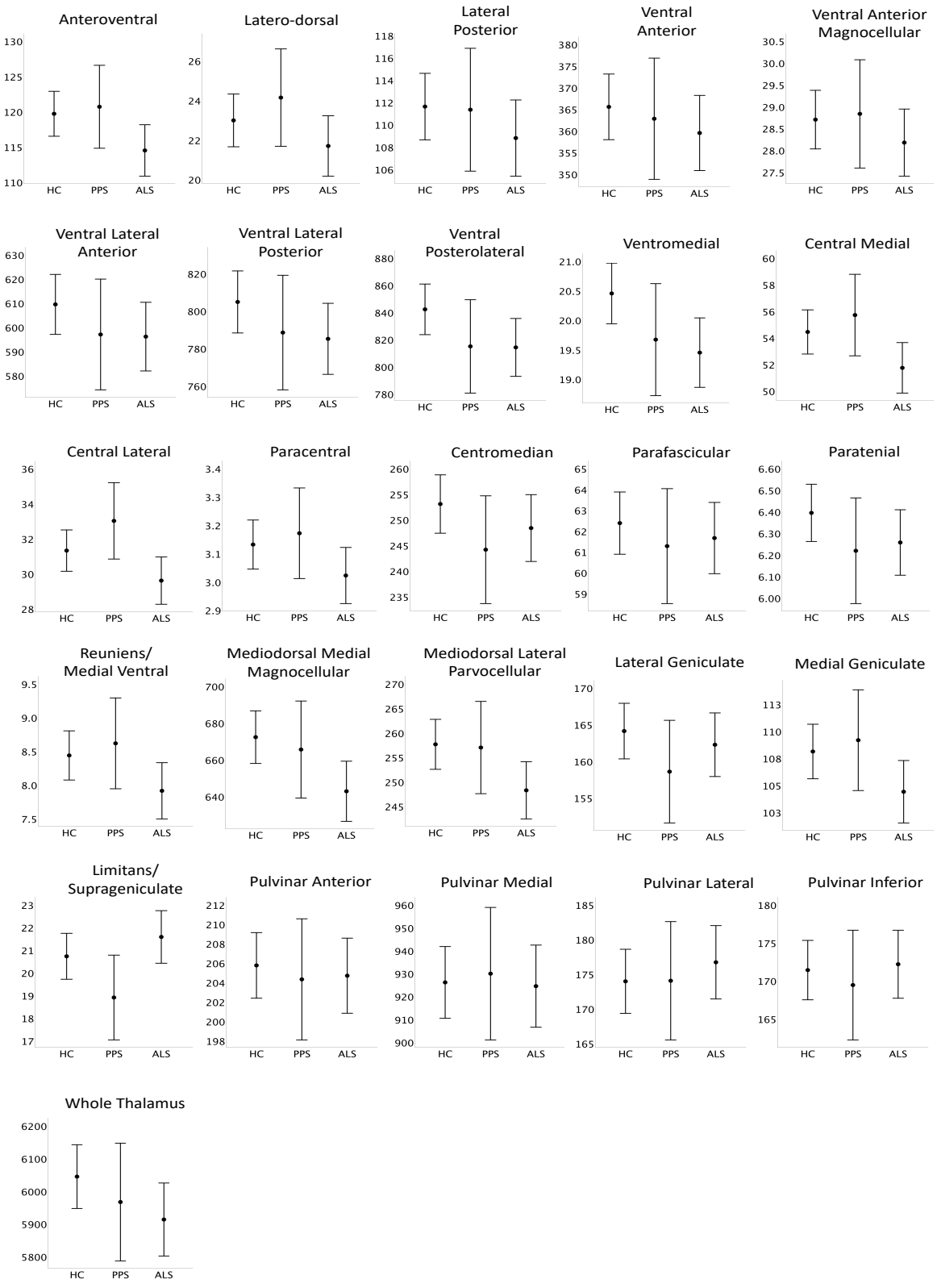

Figure 6- 3 Estimated marginal means with error bars of thalamic nuclei volumes for the 3 study groups – HC, PPS and ALS.

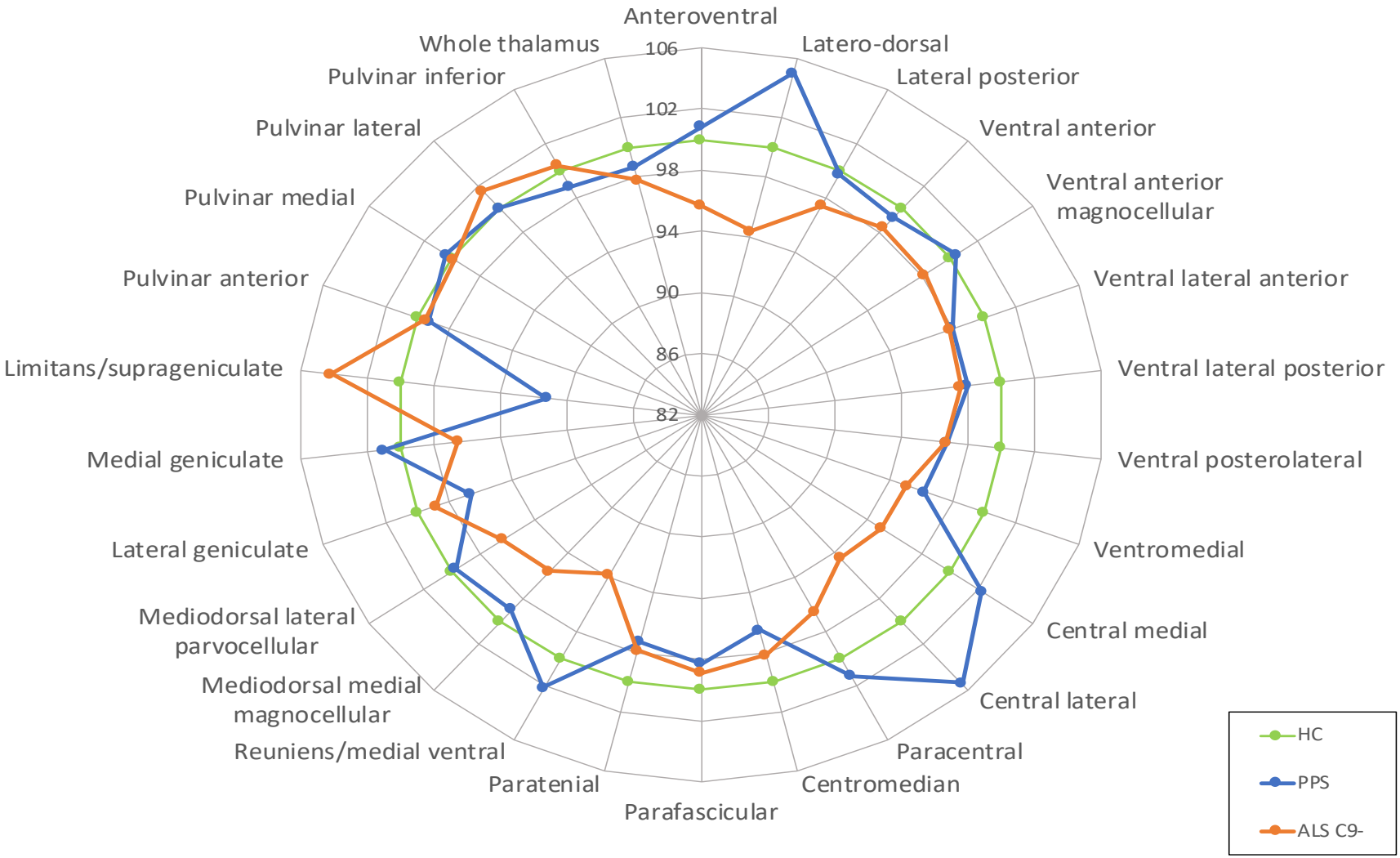

Figure 6- 4 Percentage change of each thalamic nuclei of PPS (blue) and ALS (orange) groups with respect to estimated marginal means of healthy controls (green) represented by 100%.

6.3.2 Group of thalamic nuclei based on their physiological function

Upon grouping the thalamic nuclei based on their physiological function **(Table 6-3.)**, significant atrophy was denoted in the mediodorsal-paratenial-reuniens nuclei in the ALS C9- group in contrast to healthy controls (p=.023). A trend in intergroup differences was also noted in the anteroventral nuclei (p=.066) driven by the ALS C9- group. On the other hand, our PPS group demonstrated no significant differences in reference to healthy controls or the ALS C9- group.

Thalamic nuclei	**Study group**	**EMM**	**Standard error**	**ANCOVA Sig. (*p*)**	**PPS vs HC**	**ALS C9- vs HC**	**PPS vs ALS C9-**
Anteroventral	HC	119.666481	1.616862	**.066[t]**	1.000	.110	.240
	PPS	120.661735	2.985621				
	ALS C9-	114.465955	1.850713				
Lateral geniculate	HC	164.094978	1.922915	.397	.538	1.000	1.000
	PPS	158.564826	3.550763				
	ALS C9-	162.233904	2.201031				
Medial geniculate	HC	108.110975	1.279264	.096	1.000	.177	.266
	PPS	109.156469	2.362228				
	ALS C9-	104.393179	1.464287				
Pulvinar-limitans	HC	1497.863223	12.016881	.992	1.000	1.000	1.000
	PPS	1496.525813	22.189805				
	ALS C9-	1499.540124	13.754915				
Laterodorsal	HC	22.980281	0.677146	.205	1.000	.641	.296
	PPS	24.133640	1.250387				
	ALS C9-	21.687076	0.775084				
Lateroposterior	HC	111.608194	1.515199	.452	1.000	.674	1.000
	PPS	111.327692	2.797895				
	ALS C9-	108.783497	1.734346				

Mediodorsal-paratenial-reuniens	HC	944.675752	9.619652	**.025***	1.000	**.023***	.380
	PPS	937.275863	17.763196				
	ALS C9-	905.154253	11.010969				
Motor hub	HC	1808.334614	18.273164	.346	1.000	.474	1.000
	PPS	1776.893547	33.742361				
	ALS C9-	1768.711183	20.916061				
Sensory hub	HC	862.544680	9.703694	.114	.531	.156	1.000
	PPS	834.490096	17.918382				
	ALS C9-	833.517709	11.107165				
Intralaminar	HC	404.278640	4.317554	.312	1.000	.402	1.000
	PPS	397.258514	7.972592				
	ALS C9-	394.338436	4.942014				
Whole thalamus	HC	6044.157819	49.529446	.222	1.000	.254	1.000
	PPS	5966.288194	91.458734				
	ALS C9-	5912.825316	56.693024				

Table 6- 3 Volume of thalamic nuclei grouped according to physiological function of patients with post-polio syndrome (PPS), *C9orf72* negative ALS (ALS C9-) and healthy controls (HC). Statistical tests corrected for multiple comparisons (Bonferroni), age, sex, education and TIV. * highlights $p < 0.05$ ^{t} highlights trends at $p < 0.08$.

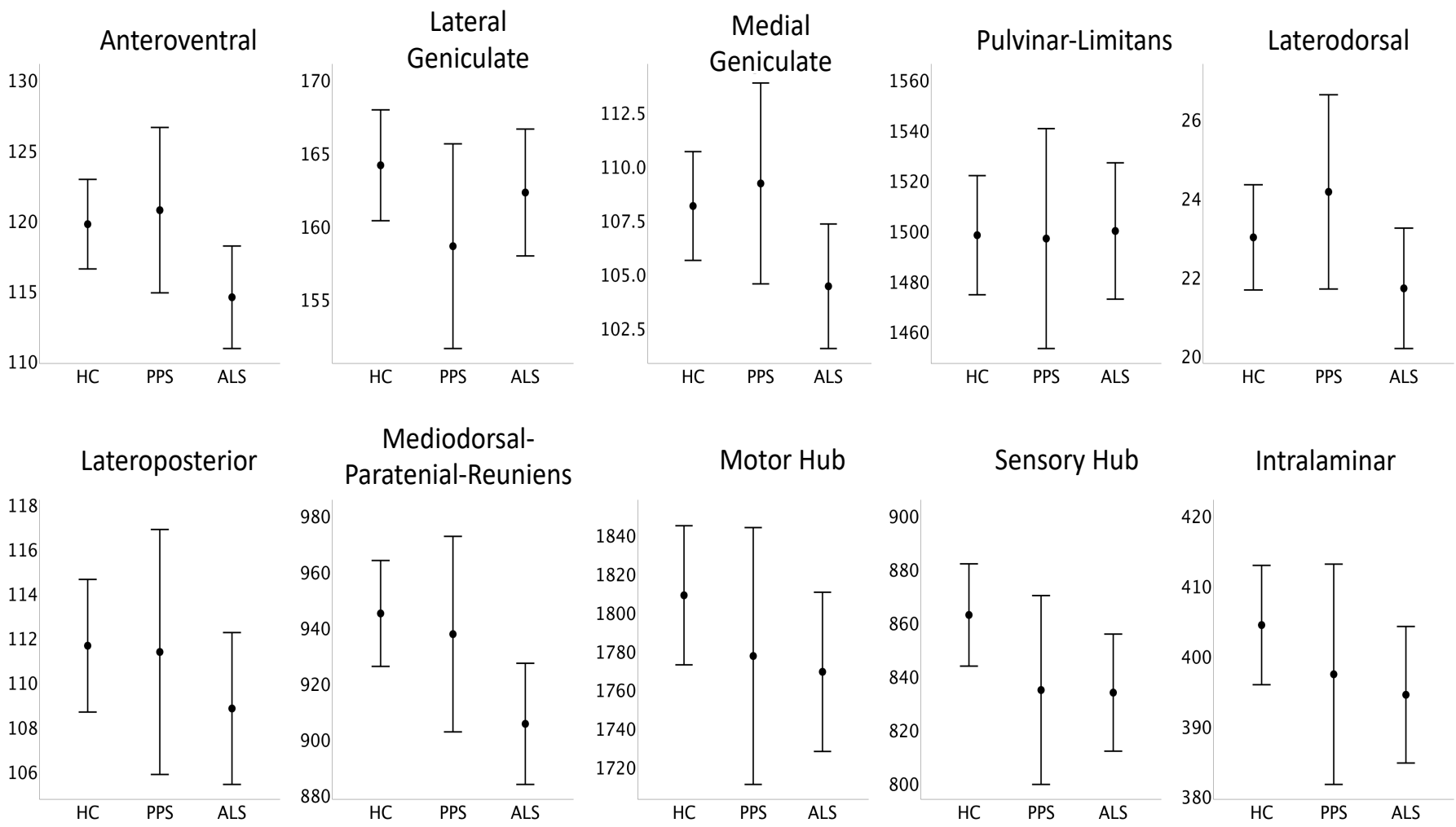

Figure 6- 5 Estimated marginal means of thalamic nuclei volumes grouped as per physiological function with error bars for healthy controls, patients with post-polio syndrome (PPS) and amyotrophic lateral sclerosis (ALS).

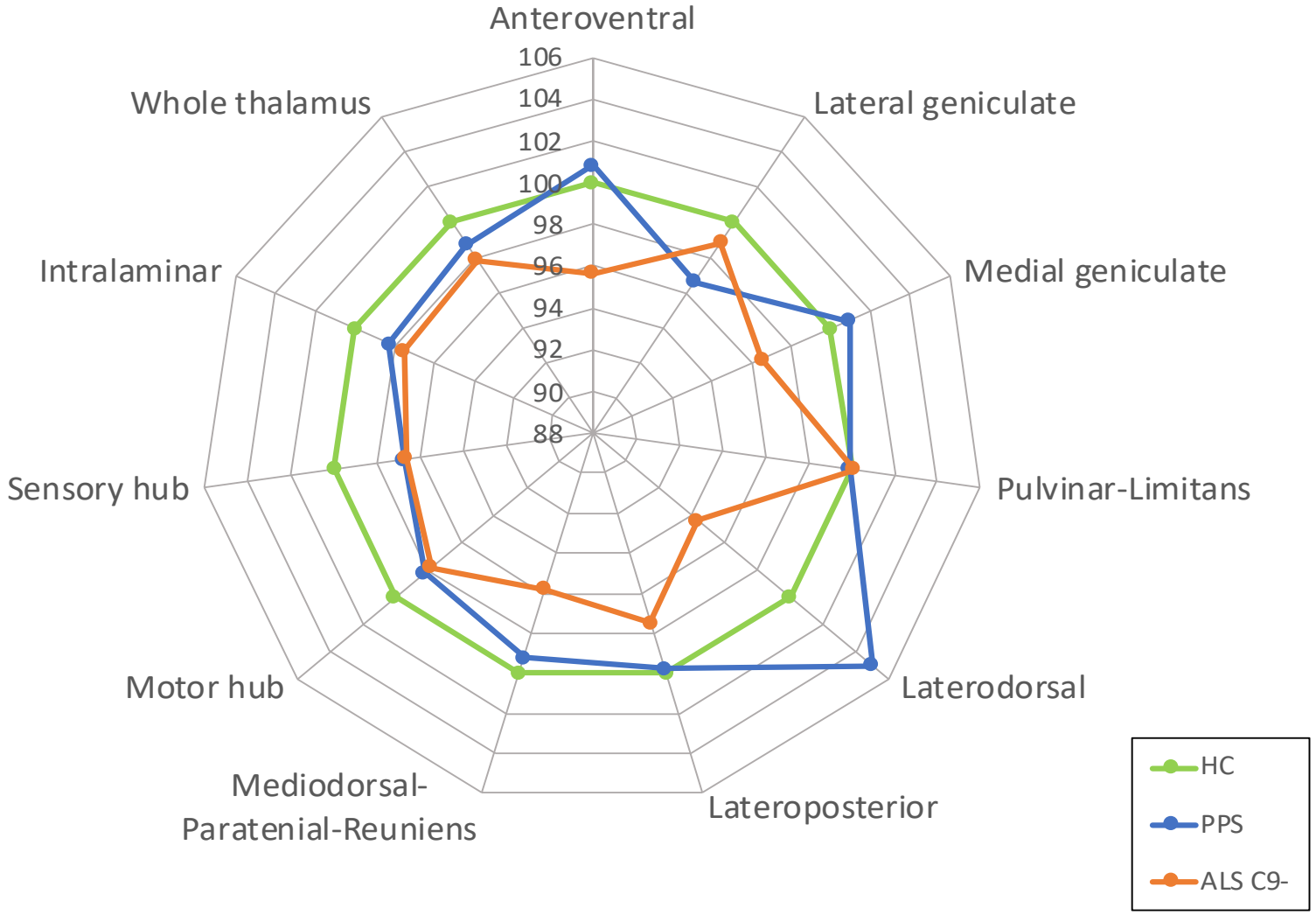

Figure 6- 6 Radar plot of percentage change of thalamic nuclei grouped based on physiological function for PPS (blue) and ALS (orange) with respect estimated marginal means of healthy controls (green) represented by 100%.

6.3.3 Amygdala

Our analyses demonstrated no significant differences between the study groups in both the amygdala as a whole ($p = .556$) and within the individual nuclei. Our PPS group showed no atrophy in neither of amygdala nuclei in contrast to healthy controls or ALS group. Conversely, no amygdala nuclei atrophy was noted in the ALS group in relation to healthy controls and PPS. **(Table 6-4)**

Amygdalar nuclei	Study group	EMM	Standard error	ANCOVA Sig. (*p*)	PPS vs HC	ALS C9- vs HC	PPS vs ALS C9-
Lateral nucleus	HC	680.556019	6.678756	.815	1.000	1.000	1.000
	PPS	680.508208	12.332676				
	ALS C9-	674.361585	7.644723				
Basal nucleus	HC	459.630357	4.727671	.526	1.000	.775	1.000
	PPS	456.387470	8.729893				
	ALS C9-	451.426941	5.411446				
Accessory basal nucleus	HC	274.666027	3.051223	.170	1.000	.188	1.000
	PPS	269.008538	5.634244				
	ALS C9-	265.922628	3.492530				
Anterior amygdaloid area	HC	56.426389	0.726510	.151	1.000	.544	.210
	PPS	57.816204	1.341539				
	ALS C9-	54.935057	0.831587				
Central nucleus	HC	46.879179	0.697284	.961	1.000	1.000	1.000
	PPS	46.805368	1.287572				
	ALS C9-	47.142907	0.798135				
Medial nucleus	HC	20.935142	0.463778	.934	1.000	1.000	1.000
	PPS	21.222645	0.856390				
	ALS C9-	20.848339	0.530855				

Cortical nucleus	HC	26.501815	0.369302	.146	1.000	.154	1.000
	PPS	25.843856	0.681935				
	ALS C9-	25.393751	0.422715				
Cortico-amygdaloid transition	HC	181.750970	2.094592	.481	1.000	.763	1.000
	PPS	178.351520	3.867774				
	ALS C9-	178.086411	2.397538				
Paralaminar nucleus	HC	51.941678	0.522833	.969	1.000	1.000	1.000
	PPS	51.709831	0.965438				
	ALS C9-	51.780760	0.598451				
Whole amygdala	HC	1799.287576	17.704367	.556	1.000	.839	1.000
	PPS	1787.653641	32.692046				
	ALS C9-	1769.898380	20.264997				

Table 6- 4 The volumetric profile of amygdala nuclei of healthy controls (HC), patients with post-polio syndrome (PPS) and *C9orf72* negative amyotrophic lateral sclerosis (ALS C9-). Statistical tests corrected for multiple comparisons (Bonferroni), age, sex, education and TIV. * highlights $p < 0.05$ ^{t} highlights trends at $p < 0.08$.

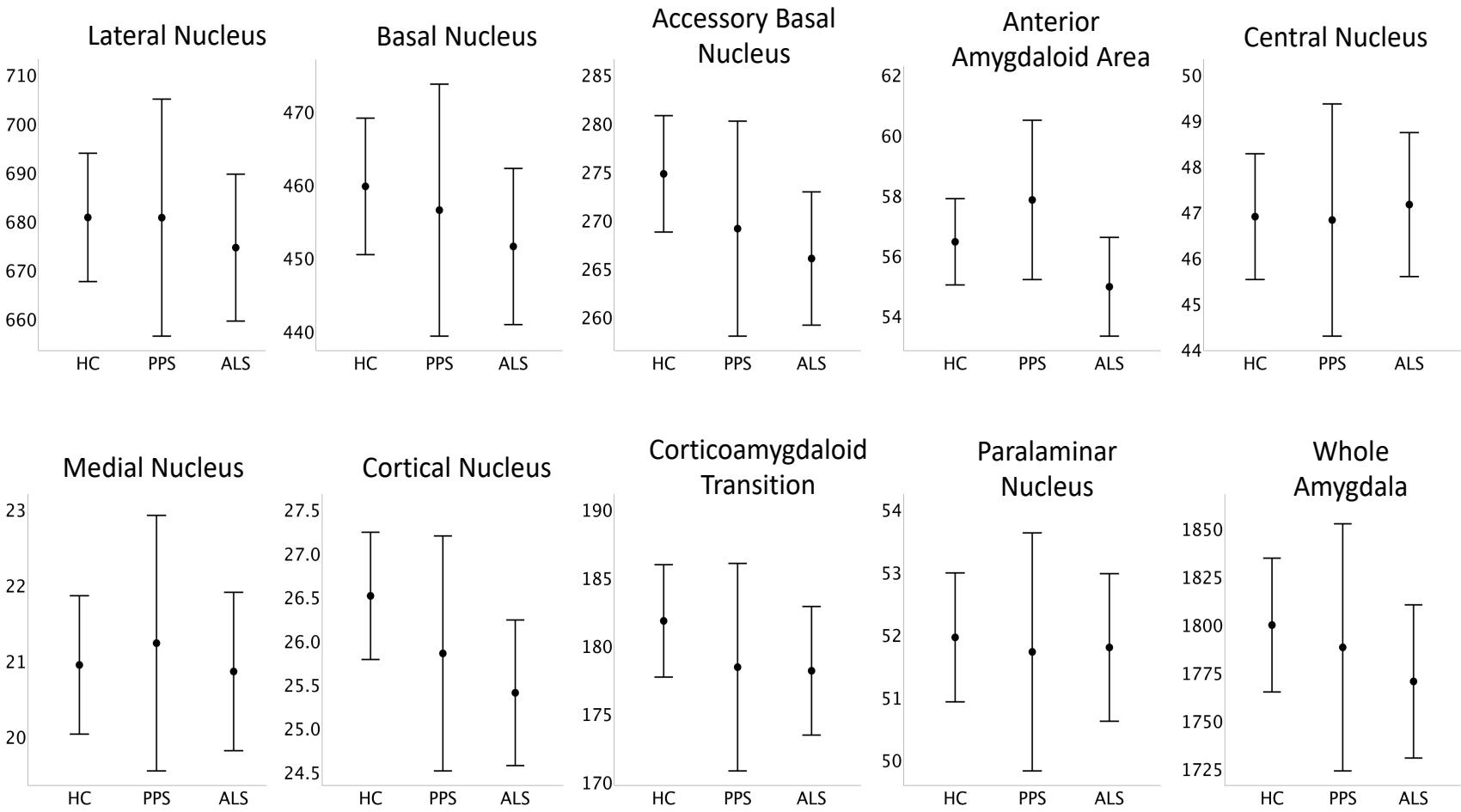

Figure 6- 7 Estimated marginal means with error bars of amydalar nuclei volumes of healthy controls (HC), patients with post-polio syndrome (PPS) and *C9orf72* negative amyotrophic lateral sclerosis (ALS).

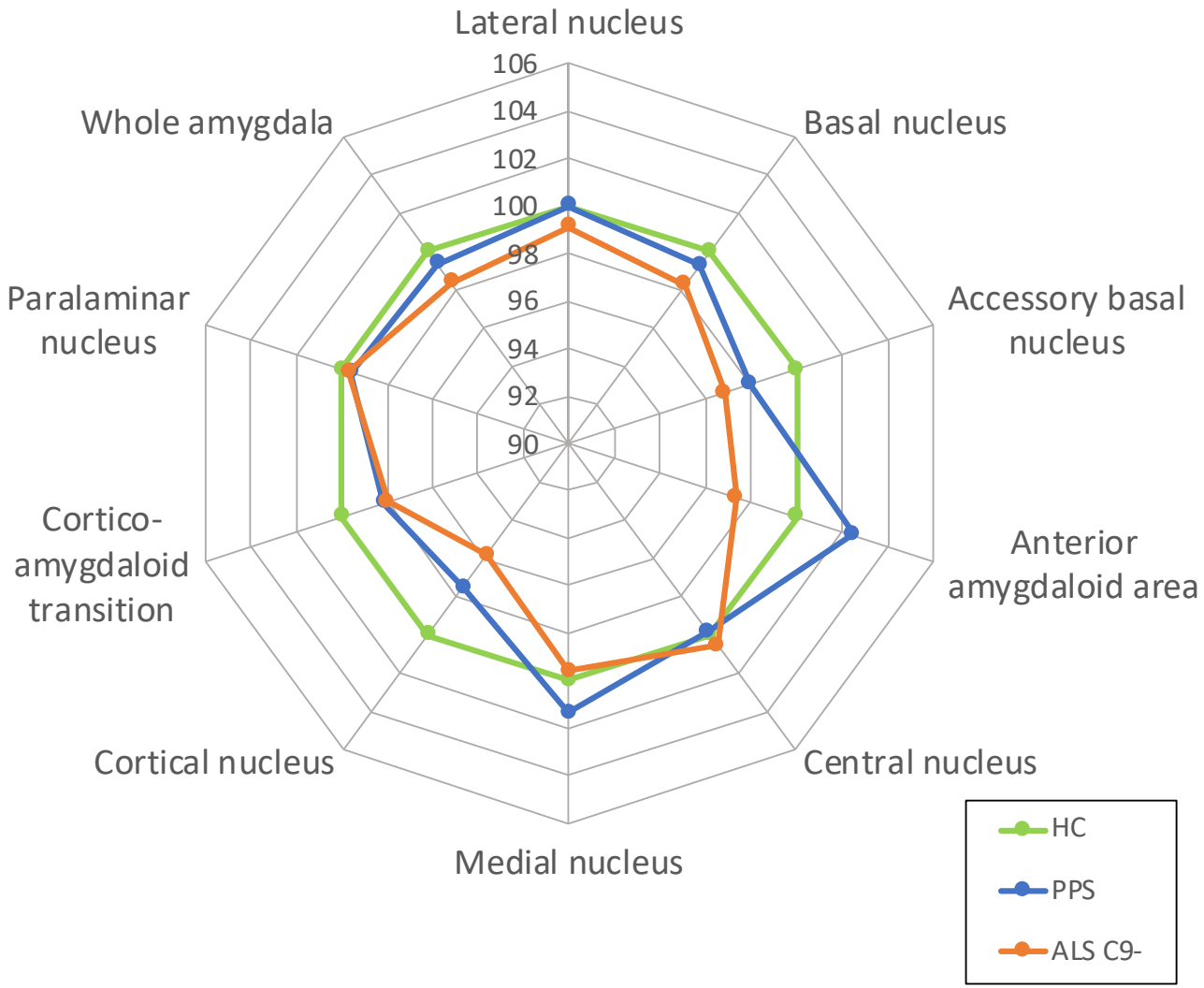

Figure 6- 8 Percentage change of amygdala nuclei volume in PPS (blue) and ALS group (orange) with respect to estimated marginal means of healthy controls (green) represented by 100%.

6.4 Discussion

The main objective of this study was the investigation and description of thalamic and amygdaloid involvement in PPS patients with the appraisal of their individual constituting nuclei, which has not been attempted previously. Our imaging findings provide robust evidence of the well-preserved structural integrity of the thalamus and amygdala in PPS despite the range of extra-motor symptoms experienced by that cohort. These observations suggest that commonly observed extra-motor clinical manifestations in PPS such as fatigue, cognitive deficits or behavioural symptoms [590] do not stem from subcortical brain structural alterations.

Generalised fatigue is one of the most frequently reported and most disabling PPS symptoms. In addition to the incapacitating physical component of fatigue, many polio patients also reported "brain fatigue" to describe cognitive impairment such as word findings difficulties, attention and concentration deficits, clouded thinking, memory deterioration and difficulty in maintaining wakefulness [83, 84, 86]. In the context of post-polio fatigue, these symptoms were hypothesised to be linked to widespread polio-induced damage to brain areas responsible for cortical activation, including the thalamus, with supporting evidence from previous post-mortem studies [49, 52] and qualitative neuroimaging studies [48]. Fatigue has also been linked to thalamic pathology in other neurological conditions such as multiple sclerosis (MS) [506, 591], neuromyelitis optica spectrum disorder (NMOSD) [592] or traumatic brain injury (TBI) [593-595] for example. Given that the thalamus is a central relay station with ample connections and underpin a wide range of functions, in addition to fatigue, the thalamus has also been implicated in cognitive impairment in a several neurological disorders including MS [596], epilepsy [597], dementia [598], stroke [599], TBI [600] or Alzheimer's disease [601]. Pulvinar nuclei has been found to play a role in selective attention [602] while the intralaminar/midline thalamic nuclei play a role in non-specific attention, arousal and awareness with its connections to the parietal lobes [603]. The anterior thalamic nucleus plays an important role in spatial learning and memory through its connections to the hippocampus [603]. The medial dorsal thalamic nucleus is implicated in declarative memory involving the use of memory strategies for information retrieval via its connections to the prefrontal cortex and limbic system [603]. However, in this study, our cohort of PPS patients did not demonstrate any atrophy within the thalamus when

evaluated as either a global structure or upon further segmentation into its distinct nuclei when contrasted to healthy controls.

Furthermore, our imaging findings demonstrated a lack of structural alterations in the amygdala as a global structure or within its individual nuclei in PPS patients when compared to healthy controls. The amygdala is a limbic structure that plays an important role in the processing and regulation of emotions, emotional encoding of memories, emotional learning, reward learning and instrumental behaviour. The lateral nucleus is essential for the fear conditioning and memory formation while the basolateral nucleus plays an integral role in anxiety. The basolateral amygdalar nuclei plays a crucial role in reward seeking processes via its projection to the nucleus accumbens. The basomedial amygdala plays a role in the reduction of fear-related freezing and high-anxiety states. While our previous neuroimaging study in PPS patients highlighted atrophy within the nucleus accumbens, our imaging findings shows that the atrophy does not extend to the amygdala.

Our findings are in line with more recent studies that showcased a lack of or very limited cortical and subcortical pathology in polio survivors in the presence of extra-motor manifestations in PPS. This is the first neuroimaging study dedicated to the systematic assessment of the thalamus and amygdala in that cohort and larger studies would be needed to validate our findings. However, the absence of thalamic or amygdala pathology in PPS is reassuring as it points towards other possible modifiable factors contributing to the PPS fatigue such as poor sleep including sleep-related breathing disorders, increased BMI, decreased cardiovascular fitness, sedentary lifestyle, subclinical or overt respiratory compromise, pain, polypharmacy or poor mental health. This provides rationale for the continuous support from the multidisciplinary team (MDT) for an individualised care tailored to the needs of each polio patient addressing both the physical and non-physical aspects of PPS to mitigate the sensation of fatigue and associated cognitive deficits.

This study is however, not without limitations. The sample size of our PPS cohort was relatively small and did not enable for further stratification of patients according to their age at initial poliomyelitis or disease burden and severity. Due to the nature of the study, there is an inherent selection bias towards participants with less severe symptoms

including fatigue, pain, cognitive and behavioural deficits that were able to attend the research facilities and tolerate the duration of the MR protocol. This study was of a cross-sectional design that only captured a snapshot of the thalamus and amygdala many decades after the initial infection rather than the longitudinal changes that may have occurred over that period of time. Lastly, it is worth noting that our study methods allowed for the in-depth evaluation of structural alterations rather than the detection of functional alterations in cortical-subcortical brain networks which could be present in the absence of subtle structural changes to explain the fatigue and the related cognitive deficits.

6.5 Conclusions

This study did not detect widespread subcortical damage in poliomyelitis survivors as previously reported. Our PPS cohort demonstrated no evidence of structural alterations in any individual thalamic nuclei or amygdalar nuclei. This likely suggests that the constellation of extra-motor symptoms such as fatigue, chronic pain, cognitive and mood disturbances may be attributed to causes other than damage to the subcortical brain regions. Future larger multi-centre studies including a larger sample size of PPS patients would be crucial to validate our findings. Additionally, functional neuroimaging data in this cohort would be valuable in assessing the dynamic recruitment and dysfunction of cortico-subcortical brain networks in PPS patients which could unravel the anatomical underpinnings of PPS symptoms.

7 Overarching conclusions

The main objective of this research project was the in-depth characterisation of the cerebral changes in poliomyelitis survivors. The practical relevance of evaluating brain changes in polio survivors is twofold; it helps to investigate the statistical substrate of clinical observations and cerebral changes in poliomyelitis survivors may serve as a model for other longstanding anterior horn pathologies irrespective of the underlying aetiology. The clinical profile of our cohort has been painstakingly evaluated using a standardised battery of clinical assessment tools so that the anatomical underpinning of extra-motor symptoms, such as fatigue, cognitive and behavioural deficits can be systematically evaluated. A variety of whole-brain and region of interest analyses were performed, a multitude of grey and white matter integrity metrics were interrogated and both cortical and subcortical structures assessed.

Adult polio survivors exhibit considerable motor disability, affecting predominantly the lower limbs. Extra-motor features can also be readily detected, including verbal fluency and language deficits, apathy and considerable fatigue. Quantitative analyses only identified limited brain atrophy in our cohort of poliomyelitis survivors restricted to the cingulate gyrus and left nucleus accumbens. Conversely, adult polio survivors exhibited cortical hypertrophy compared to controls in the brainstem, cerebellum and occipital lobe. This was complemented by superior white matter organisation in the corticospinal tracts, cerebellum, bilateral mesial temporal lobes and inferior frontal regions as well as within cerebellar peduncular tracts. The increased grey and white matter integrity indices observed in this cohort decades after the original infection may potentially be interpreted as structural reorganisation in response to severe lower motor neuron insult in the early years of life.

7.1 Relevance and significance of findings

Despite the large number of polio survivors currently living with the lasting effects of the acute paralytic infection around the globe, there has been a striking paucity of research studies investigating this unique population. Post-polio syndrome remains currently a diagnosis of exclusion and the mainstay treatment includes lifestyle modifications, strategies to conserve energy while preventing further muscle loss and symptomatic relief

and no current disease-modifying therapy exists. There is a lack of diagnostic, prognostic and monitoring biomarkers for this slowly progressive neurological entity which hinders the design of new clinical trials. Despite the rarity of acute poliomyelitis today, it is likely to persist for decades to come especially in countries where the poliovirus was endemic until recently or is still currently endemic. The relevance and implications of characterising the cerebral radiological profile of this unique cohort is not limited to polio survivors but may have implications to the wider spectrum of lower motor neuron conditions and spinal cord conditions, in both adult and paediatric populations.

Our main finding is that adult polio survivors exhibit widespread cerebral GM hypertrophy and increased WM integrity compared to healthy controls. GM changes were detected in the brainstem, occipital lobe and cerebellum. Specifically, hypertrophic changes were detected in cerebellar lobules associated with sensorimotor functions, notably the lobules I, II and III of anterior lobe and lobules VIIIA and VIIIB from the posterior lobe. Well-organised WM tracts were noted along the corticospinal tracts, bilateral mesial temporal lobes, inferior frontal areas and within the cerebellum, including the cerebellar peduncular tracts. These findings may putatively be interpreted as structural cortical and cerebellar reorganisation owing to neuroplasticity, in response to the spinal cord insult early in life, potentiated by extensive motor and cognitive training. It is conceivable that similar cerebral reorganisation or adaptive processes take place in other infective, inflammatory, vascular or neurodegenerative spinal conditions. The ascertainment of increased cortical thickness and increased cerebral white matter integrity metrics may also justify arduous rehabilitation efforts in spinal insults sustained in childhood.

Considerable extra-motor symptom burden was detected in this cohort of adult polio survivors. A high prevalence of apathy, verbal fluency and language deficits and problematic fatigue were observed. In addition, deficits in executive function, memory and visuospatial domains were also captured. From the clinical perspective, these findings are very important to take into consideration in the management, resource allocation and enrolment of these patients in clinical trials. As adult polio survivors is an ageing population, they are particularly prone to develop other serious secondary comorbidities [604] such as hypertension, cardiorespiratory and vascular disease such as ischaemic heart disease, hypercholesterolaemia, varicose veins but also joint diseases and osteopenia

with decreasing muscle mass, making them susceptible to be on multiple prescription medications and vulnerable to unwanted side effects. Due to comorbid cognitive impairment, they may be less likely to adhere to medications or rehabilitation efforts [605]. Furthermore, the high level of debilitating fatigue and apathetic behaviour experienced by that cohort will have further implications in engaging in social activities and finding pleasure in previously enjoyed activities, maintaining employment, fulfilling certain duties and responsibilities, maintain self-care all of which further hampers their sense of self-worth, dignity, increases the feeling of guilt and worthlessness which in turn can lead to isolation and depression. As such it might be difficult in maintaining motivation and would negatively impact their participation in their rehabilitative care or in new clinical trials that could be of potential benefit to them.

Our findings suggest that the considerable extra-motor manifestations may not stem from widespread cortical or subcortical atrophy as previous suggested. Atrophy is limited to the cingulate gyrus, temporal poles and left nucleus accumbens which could account for the apathy observed in this cohort. These findings are reassuring as it suggests alternative, non-structural causes, which may be driving the multitude of neuropsychological manifestations experienced by these patients. These factors are likely to be multifactorial encompassing a combination of polypharmacy, poor sleep, increased BMI, decreased cardiorespiratory fitness, subclinical or overt respiratory compromise, chronic pain and low mood rather than focal brain pathology. The notion that the extra-motor symptoms may be primarily caused by modifiable factors, can potentially inform individualised multidisciplinary interventions. Expert support from physiotherapy, occupational therapy, mental health support, speech and language therapy, pain specialist, social care input, nurse specialist and orthotists can be tailored to each patient individually. Our findings are based on a single-centre, clinic-based study. Setting up national polio registries, active patient advocacy and dedicated conferences on this condition may not only raise awareness of this debilitating condition but would help to validate these findings and lead to consensus management strategies.

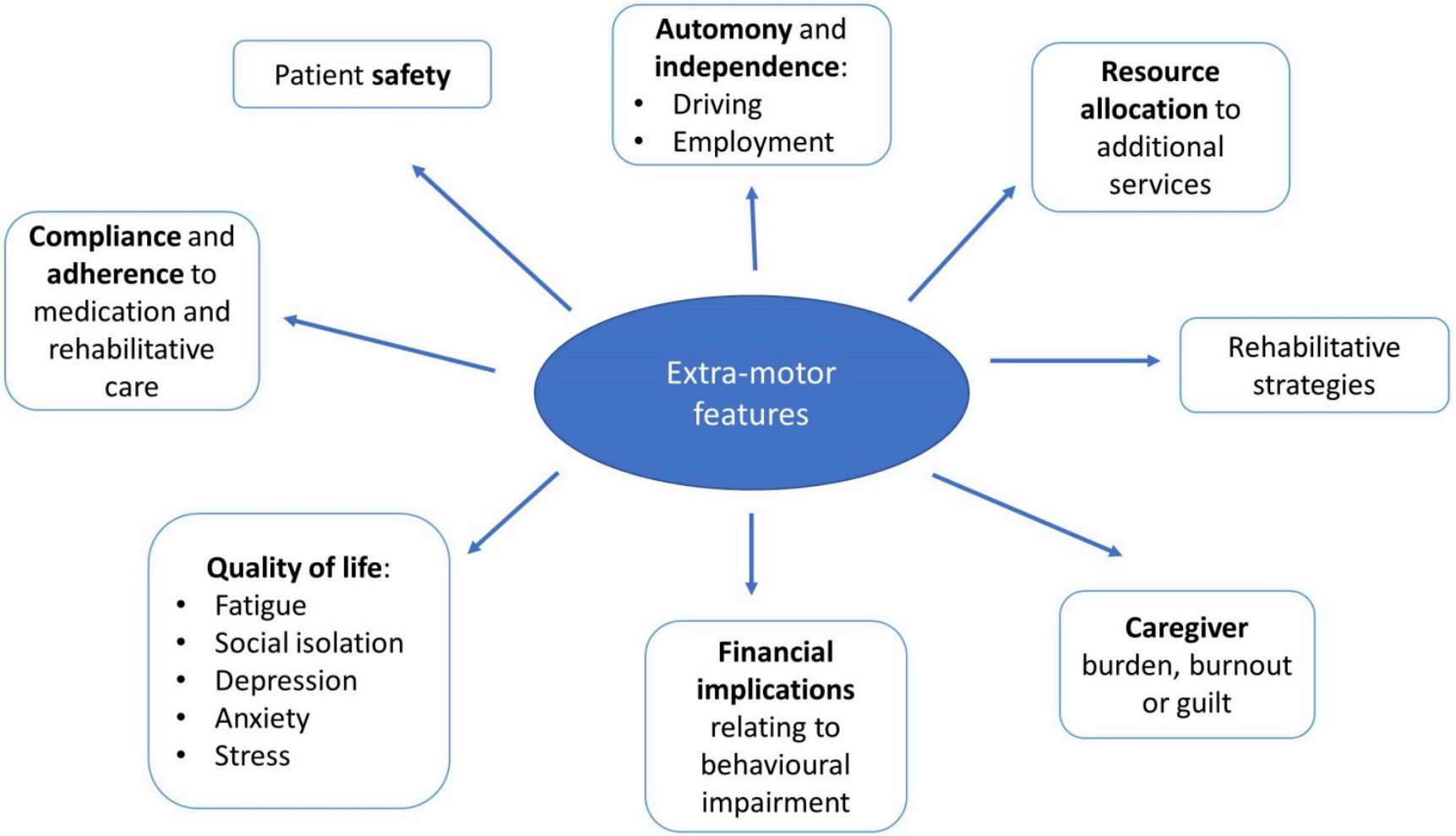

Figure 7- 1 The relevance and implications of extra-motor involvement in polio survivors.

From an academic point of view, adult polio survivors may also serve as a template for slowly progressive lower motor neuron disease such as SMA. Our findings help to map brain regions that are susceptible to neuroplasticity and highlight the potential for structural MRI to detect compensatory processes which is rarely captured [419, 560].

7.2 Limitations of study

This research study was not without limitations. As a result of the ageing population of poliomyelitis survivors in Europe, recruitment was intrinsically challenging. For MRI safety reasons, patients with recent joint replacements and incompatible implanted medical devices to the MRI scanner were also excluded from participating in this study. Due to the nature of the study, participants were required to attend the research facilities to undergo the MR images acquisition. However, with the high level of functional disability affecting the lower limbs in this cohort, mobility is exceptionally impaired. As a result, patients who were unable to travel to the research facilities were not recruited which further constricted the sample size. Furthermore, only participants who were able to tolerate the duration of the MR imaging protocol were recruited. This therefore excluded those with severe disease burden such as severe spinal deformities such as severe kyphoscoliosis and significant respiratory or bulbar impairments. Not only did this reduce the number of poliomyelitis survivors recruited but also suggested that the findings of this study were representative of a subset of poliomyelitis survivors with milder symptoms rather than the broader population of poliomyelitis survivors. Recruitment was further hampered as a result of COVID-19 pandemic which slowed down data collection.

With the limited literature on post-polio syndrome and the striking paucity of quantitative neuroimaging studies, it was not possible to compare and contrast our imaging findings observed here in an Irish population of poliomyelitis survivors to different group of poliomyelitis survivor from another part of the world or validate our findings against post mortem studies. Lastly, while the utilisation of multiple imaging analyses methodologies such as whole-brain and region-of-interest analyses, voxel-based morphometry, tract-based spatial statistics, cortical thickness and volumetric analyses enabled the nuanced characterisation of structural GM and WM changes in poliomyelitis survivors, the use of

additional imaging modalities could provide further insights into both pathological and compensatory processes in this population.

7.3 Implications for future research

The findings of this research study have provided evidence of limited structural degeneration and detectable cerebral reorganisation in a group of patients with longstanding lower motor neuron pathology. This work expanded the literature of extra-motor manifestations in post-polio syndrome. With the absence of widespread brain atrophy as the primary cause of extra-motor manifestations, the aetiology of post-polio fatigue remains to be elucidated. Fatigue refers to a multitude of experiences such as 'lack of energy', reduced physical endurance, physical discomfort, lack of motivation and sleepiness but also word findings difficulties, poor concentration, limited attention span, difficulty in maintaining wakefulness and mood disturbances. Generalised fatigue remains one of the most debilitating symptoms of polio survivors and is likely to be multifactorial owing to muscle unit pathology, weight-gain, respiratory compromise, polypharmacy, cardiorespiratory deconditioning, poor sleep and chronic pain. Future research in this cohort should specifically evaluate the impact of chronic pain, the incidence, severity and effect of sleep disorders, screen for overt or subclinical respiratory compromise, include comprehensive multi-domain neuropsychological battery, use multi-dimensional fatigue assessments, test for mood disturbances and comprehensively assess the impact of this disease on emotional, social, educational, employment and financial aspects of life. Additionally, the use of functional neuroimaging such as fMRI would be beneficial to gain a better insight into brain network activation in polio survivors.

Our findings demonstrate cerebral alterations in what was traditionally regarded as a lower motor neuron condition. This provides evidence of the importance of investigating cerebral changes in LMN-dominant conditions rather than the assumption that there is an inherent sparing of supraspinal regions. Additionally, with the emerging methods to overcome the challenges associated with spinal cord imaging [58, 606], studies including both cerebral and spinal cord MR protocols would be beneficial in having a better overlook at the interaction between the spinal cord and cerebrum. There are already studies that have undertaken this [419]. This will allow for the simultaneous ascertainment of spinal pathology with concurrent cerebral changes to confirm if adaptive processes take place along the neuroaxis.

Poliomyelitis survivors demonstrated GM hypertrophy and increased WM integrity affecting various cortical areas and focal cerebellar areas associated with sensorimotor functions, all interpreted as a compensatory mechanism. As functional neuroimaging studies have been observed to more readily capture hyperactivation or recruitment of additional brain regions during specific tasks in MND to demonstrate compensatory mechanisms, these results indicate that multimodal neuroimaging studies involving a combination of structural MRI, functional MRI, magnetic resonance spectroscopy or arterial spin-labelling perfusion MRI may be beneficial. Additionally, there is a likely bias towards reporting atrophic changes within the brain or spinal cord as a sign of pathology when compared to healthy controls potentially overlooking structural compensatory changes in motor neuron diseases. Therefore, our research suggests that it is also important to be mindful of the possibility of compensatory mechanisms. Furthermore, the findings of this thesis are based on a prospective, single-centre cross-sectional observational study. It would be important to undertake large multi-centre studies with a longitudinal design to evaluate progressive changes that may occur.

Methodology of this study	Main findings of this research study	Priorities for future studies
• Prospective, cross-sectional observational study • Inclusion of healthy control group (HC) and amyotrophic lateral sclerosis (ALS) as disease control group • Battery of clinical assessments including structured interview, physical neurological examination, questionnaires: ALSFRS-R, ECAS, HADS, FrSBe, FSS, PFS, Penn UMN score • Structural and Diffusion tensor MRI • Cortical GM analyses: VBM, cortical thickness • Cortical WM analyses: Tract-based statistics • Subcortical analyses: volumetry • Cerebellar analyses: cortical thickness, volumetry, morphometry, cerebellar peduncles • Thalamic analyses: volumetry • Amygdala analyses: volumetry	• LMN symptoms and signs and high functional disability especially in lower limbs but also affect upper limbs, bulbar and respiratory areas • Extra-motor symptoms with high prevalence of fatigue, verbal fluency and language deficits and apathy • Lack of widespread cortical, subcortical and cerebellar atrophy • Cortical GM hypertrophy in brainstem, occipital lobe, cerebellum • Increased WM integrity in CST, cerebellum, mesial temporal lobes, inferior frontal regions • Cingulate gyrus and left nucleus accumbens atrophy • Cerebellar lobules hypertrophy in lobules I,II, III and VIIIA/B accompanied by increased WM integrity in middle cerebellar peduncles • Absence of degenerative changes in thalamic or amygdalar nuclei.	• Prospective, longitudinal multi-centre population-based observational study • Standardised clinical assessments to investigate other contributing factors to post-polio fatigue; e.g pain, poor sleep, social impact and feedback from caregiver • Dual spinal cord and brain MRI • Multimodal neuroimaging studies including functional neuroimaging modalities in combination with structural MRI

Table 7- 1 Main study findings and priorities for future research.

7.4 Conclusions

Considerable motor disability was observed in poliomyelitis survivors predominantly affecting the lower limbs. A multitude of stereotyped extra-motor manifestations were ascertained including fatigue, verbal fluency and language deficits and apathy. Our quantitative radiological analyses did not identify widespread supraspinal atrophy. To the contrary, hypertrophic grey matter changes and increased white matter integrity were detected in multiple cortical and cerebellar regions. These may be interpreted as the result of adaptive processes, compensating for severe spinal cord insult early in life.

8 References

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