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REVIEW

The presymptomatic phase of amyotrophic lateral sclerosis: are we merely scratching the surface?

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Abstract

Presymptomatic studies in ALS have consistently captured considerable disease burden long before symptom manifestation and contributed important academic insights. With the emergence of genotype-specific therapies, however, there is a pressing need to address practical objectives such as the estimation of age of symptom onset, phenotypic prediction, informing the optimal timing of pharmacological intervention, and identifying a core panel of biomarkers which may detect response to therapy. Existing presymptomatic studies in ALS have adopted striking different study designs, relied on a variety of control groups, used divergent imaging and electrophysiology methods, and focused on different genotypes and demographic groups. We have performed a systematic review of existing presymptomatic studies in ALS to identify common themes, stereotyped shortcomings, and key learning points for future studies. Existing presymptomatic studies in ALS often suffer from sample size limitations, lack of disease controls and rarely follow their cohort until symptom manifestation. As the characterisation of presymptomatic processes in ALS serves a multitude of academic and clinical purposes, the careful review of existing studies offers important lessons for future initiatives.

Keywords Amyotrophic lateral sclerosis · Frontotemporal degeneration · Presymptomatic · Neuroimaging

Abbreviations

ACE-R	Addenbrooke's Cognitive Examination-Revised
AD	Alzheimer's disease
AD	Axial diffusivity
ALLFTD	ARTFL–LEFFTDS Longitudinal Frontotemporal Lobar Degeneration
ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale-revised
ANG	Angiogenin
APEX1	Apurinic/apyrimidinic endodeoxyribonuclease 1
APOE4	Apolipoprotein E4
APP	Amyloid precursor protein
ASCA	Amnestic Comparative Self-Assessment
ASO	Antisense oligonucleotide
AUC	Area under the receiver operator characteristic curve
AVLT	Auditory verbal learning test
BADL	Basic activities of daily living
BDI	Beck Depression Inventory
BNT	Boston naming test
bvFTD	Behavioural variant FTD
CBD	Corticobasal degeneration
C-CFT	C-Labeled 2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane
C-CFT	C-Labeled 2-β-carbomethoxy-3-β-(4-fluorophenyl)tropane
C-PiB	C-Pittsburgh compound B
C9orf72	Chromosome 9 open reading frame 72
CAPG	Macrophage-capping protein
CBF	Cerebral blood flow
CBI-R	Cambridge Behavioural Inventory revised
CDR	Clinical Dementia Rating Scale
CDR-SUM	Clinical Dementia Rating sum of box score
CHI3L1	Chitinase 3-like protein 1

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CHI3L2	Chitinase 3-like protein 2
CHIT1	Chitotriosidase-1
CHMP2B	Charged multivesicular body protein 2b
CMAP	Compound muscle action potential
Cr	Creatine
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CST	Corticospinal tract
CVLT	California Verbal Learning test
D-KEFS	Delis–Kaplan Executive Function System
DCTN	Dynactin subunit 1
DRS	Dementia rating scale
DTI	Diffusion tensor imaging
E/I	Excitation/inhibition
ECAS	Edinburgh Cognitive and Behavioural ALS Screen
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-linked immunosorbent assays
EMG	Electromyography
EXAMINER	Executive Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research
F-FDG	Fluorodeoxyglucose
FA	Fractional anisotropy
FAB	Frontal assessment battery
fALFF	Fractional amplitude of low frequency fluctuation
FBB	Florbetaben, a fluorine-18
FBI	Frontal Behavioural Inventory
FC	Functional connectivity
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
FTD-CDR	FTD-specific Clinical Dementia Rating
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma
FVC	Forced vital capacity
GENFI	Genetic Frontotemporal dementia Initiative
GM	Grey matter
GPNMB	Glycoprotein non-metastatic B
GRN	Progranulin
HADS	Hospital Anxiety and Depression Scale
HD	Huntington's disease
HFE	High FE2 +
HIST1H2B	Histone cluster 1, H2b
HIST1H4A	Histone cluster 1, H4
HTT	Huntingtin
i-TRAQ	Isobaric tags for relative and absolute quantitation
IADL	Instrumental activities of daily living
LDST	Letter Digit Substitution Test
LEFFTDS	Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects
LMN	Lower motor neuron
LRRK2	Leucine Rich Repeat Kinase 2
MAP2	Microtubule-associated protein 2
MAPT	Microtubule-associated protein tau
MD	Mean diffusivity
MDRS	Mattis Dementia rating scale
MEG	Magnetoencephalography
MEP	Motor evoked potential
MMSE	Mini Mental State Examination
MOCA	Montreal Cognitive Assessment
MRC	Medical Research Council rating scale
MRI	Magnetic resonance imaging
MRM	Multiple reaction monitoring
MRS	Magnetic resonance spectroscopy
mtDNA	Mitochondrial DNA
MUNE	Motor Unit Number Estimation
MUNIX	Motor Unit Number Index
MVIC	Maximal voluntary isometric contraction
Myo	Myo-inosito
NAA	N-Acetylaspartate
NEFH	Neurofilament heavy
NEFL	Neurofilament light
NEFM	Neurofilament medium;
NfL	Neurofilament light
NODDI	Neurite orientation dispersion and density imaging
NPI-Q	Neuropsychiatric Inventory Questionnaire
NPTXR	Neuronal pentraxin receptor
OPTN	Optineurin
PD	Parkinson's disease
PET	Positron emission tomography
PINK1	PTEN-induced kinase 1
PLS	Primary lateral sclerosis
pNfH	Phosphorylated neurofilament heavy chain
PON	Paraoxonase
Pre-Fals	Pre-familial amyotrophic lateral sclerosis
PREV-DEMALS	Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis Study Group
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRPH	Peripherin
PSEN1	Presenilin 1

PSEN2	Presenilin 2
RAVLT	Rey Auditory Verbal Learning Test
RBMT	Rivermead Behavioural Memory test
rCMA	Rostral cingulate motor area
RD	Radial diffusivity
ReHo	Regional Homogeneity
rs-fMRI	Resting state Fmri
RWT	Phonematic Regensburger Wortflüssigkeits-test
SAT	Semantic Association Test
SDMT	Symbol digit modalities test
SEA	Social Cognition and Emotional Assessment
SETX	Senataxin
SICI	Short interval intracortical inhibition
SIGMAR1	Sigma non-opioid intracellular receptor 1
SMA	Spinal muscular atrophy
SMN	Survival motor neuron
SNCA	Synuclein alpha
SOD1	Superoxide dismutase 1
SOP	Standard operating procedure
SPG	Spatacsin
TARDBP	TAR DNA-binding protein, 43
TBM	Tensor-based morphometry
TMS	Transcranial magnetic stimulation
TMT	Trail making test
UBQLN2	Ubiquilin-2
UCHL1	Ubiquitin carboxyl-terminal hydrolase 1
UDSNB	Neuropsychological battery of the Uniform Data Set
UMN	Upper motor neuron
UPDRS	Unified Parkinson's Disease Rating Scale
VAPB	Vesicle-associated membrane protein-associated protein B/C
VAT	Visual Association Test
VBM	Voxel-based morphometry
VCP	Valosin containing protein
VOSP	The visual object and space perception battery
WAIS	Wechsler Adult Intelligence Scale
WCST	Wisconsin Card Sorting Test
WM	White matter
WRAT	Wide Range Achievement Test

Introduction

One of the latest developments in amyotrophic lateral sclerosis (ALS) is the emergence of genotype-specific pharmacotherapies heralding a paradigm shift from generic neuroprotective strategies to precision, genotype-specific interventions [1, 2]. The fundamental heterogeneity of the disease is now universally recognised and genotype- and phenotype-specific clinical traits, radiological signatures and disease trajectories have been characterised [3–5]. The notion that a long presymptomatic phase precedes symptom manifestation and that neurodevelopmental factors may contribute to the pathogenesis of ALS is increasingly accepted [6]. The presymptomatic phase of ALS has been relatively arcane until the publication of seminal presymptomatic papers which confirmed considerable pathological changes years before symptom manifestation [7, 8]. Existing presymptomatic studies in ALS invariably suffer from sample size limitations and are strikingly diverse with regards to their study design, methodological approach and conclusions. The systematic review of these papers, the frank discussion of their limitations, and the careful integration of their findings is particularly timely with the emergence of genotype-specific therapies [1, 2]. One of the key contributions of recent imaging studies in ALS is the confirmation that by the time patients fulfil diagnostic criteria for ALS, considerable disease burden can already be detected, limiting the therapeutic potential of putative disease-modifying drugs. These observations suggest that the optimal therapeutic window is likely to be at an earlier stage in high risk, genetically susceptible populations. The presymptomatic phase of ALS has long been of academic interest [9], and inspired small-scale studies [10], dedicated terminology [9], but the advent of antisense oligonucleotide therapies (ASOs) highlights the urgency for large presymptomatic studies and the meticulous integration of molecular, pathological and radiological observations in asymptomatic mutation carriers. ASO-mediated drugs have already been approved by the US Food and Drug Administration for the treatment of spinal muscular atrophy and Duchenne muscular dystrophy [11–13], and currently being trialled for ALS [1]. The nuanced characterisation of pathophysiological processes before perceptible disability develops may help to identify the optimal therapeutic window for pharmacological intervention before irreversible functional impairment ensues. Longitudinal studies of mutation carriers spanning from the adolescence to significant disability would provide an opportunity to describe anatomical patterns of disease spread, validate current staging systems, evaluate prognostic indicators and test prevailing propagation theories such as corticofugal spread, network-wise propagation, selective vulnerability, trans-synaptic spread etc., [9, 14–16]. Reports of considerable presymptomatic cerebral pathology without overt functional impairment also suggest a degree of 'motor reserve', network redundancy or possible compensatory processes to maintain function until a critical threshold is reached and symptoms develop. Large presymptomatic studies also permit the systematic assessment of the sensitivity profile of

our current biomarkers. For example, detecting white matter changes in a patient with significant disability may be less challenging than capturing early asymptomatic changes in white matter integrity decade before projected symptom manifestation. Despite the dual academic and clinical relevance of characterising the presymptomatic course of ALS [17–19], striking inconsistencies exist in the current literature due to the sample size limitations and methodological differences [20–22]. Our objective is the careful integration of the lessons of existing presymptomatic studies in ALS, to identify key learning points from individual studies, reflect on common methodological shortcomings and distil a robust framework for future studies.

Methods

A formal systematic literature review was conducted using the following search terms on PubMed: "amyotrophic lateral sclerosis", "motor neuron disease", "*C9orf72*", "frontotemporal lobar degeneration", "frontotemporal dementia" combined with each of the following keywords "presymptomatic", "premanifest", "asymptomatic". An additional search combined the above search terms with the following keywords: "magnetic resonance imaging", "MRI", "positron emission tomography", "PET", "electromyography", "neuroimaging", "electrophysiology", "neurophysiology", "transcranial magnetic stimulation", "motor unit number estimation", "motor unit number index", "neurofilament", "biomarkers". Only original research papers were systematically reviewed. Conference abstracts published in supplements of neuroscience journals were not considered. Only human studies were systematically reviewed. No exclusion criteria were set based on year of publication, but only articles written in English were reviewed. Animal studies, review papers, opinion pieces, editorials, case reports, and case series were excluded. Based on the above criteria a total of 48 original research papers were reviewed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations. The identified papers were systematically reviewed based on the following criteria:

(1) Core study design parameters (target cohort: pre-symptomatic/pre-manifest, control group: healthy controls/non-carrier relatives/disease controls, sample size, cross sectional/longitudinal, prospective/retrospective, multi-centre/single centre, follow-up interval, number of follow-up time points, number of participants, attrition rates, follow-up into the symptomatic phase)
(2) Clinical and laboratory assessments (Genetic testing, demographic profiles, availability of electrophysiological assessment, neurological or neuropsychological data, functional rating scales)
(3) Neuroimaging methods (cerebral/spinal, whole-brain/region-of-interest, MRI / PET, structural/diffusion/spectroscopy/rs-fMRI, field strength 1,5 T/3 T / 7 T, single/multi-modal analyses, post-mortem validation.

To appraise methodological approaches in other neurodegenerative conditions and their potential applicability to ALS, a selection of presymptomatic imaging papers were also reviewed in frontotemporal dementia (20 papers), Huntington's disease (11 papers), Alzheimer's disease (13 papers) and Parkinson's disease (13 papers).

Results

While the terms 'preclinical', 'premanifest' and 'presymptomatic' are widely used, the term 'asymptomatic mutation carrier' is preferred by many. It has been proposed [20] that term 'preclinical' should be reserved for the period where there are no identifiable pathological changes and the term 'presymptomatic' used for the phase when neuroimaging, electrophysiology or detailed cognitive assessment may already detect abnormalities. Despite these recommendations the above terms are often used interchangeably. The number of papers identified stratified by the key study methodology are shown in Fig. 1.

Neuroimaging studies of asymptomatic mutation carriers

Cohort characteristics

Based on our search criteria, we have identified twenty-five imaging studies investigating presymptomatic *C9orf72* hexanucleotide carriers [7, 8, 23–30], four studies focusing on presymptomatic *SOD1* carriers [10, 30–35], and three studies evaluating both [30–32]. We also identified a study which included *NEK1*, *TARDBP* and *FUS* gene mutation carriers in addition to participants with the *C9orf72* and the *SOD1* [31]. The number of presymptomatic subjects included in single studies showed significant variation ranging from 2 [35] to 249 [36]. Eight studies had included over 100 presymptomatic carriers [36–42]. The number of presymptomatic *C9orf72* carriers included ranges from 2 [32] to 83 [28] and the number of presymptomatic *SOD1* carriers ranges from 2 [35] to 24 [10] in the current literature. Thirteen studies also included symptomatic patients [28, 40, 43–45], and 19 studies only focussed their investigation on presymptomatic cohorts [8, 27, 29, 37, 38]. The strategy to select symptomatic participants was inconsistent; some studies only included symptomatic gene carriers [10, 23, 24, 28, 31, 35,

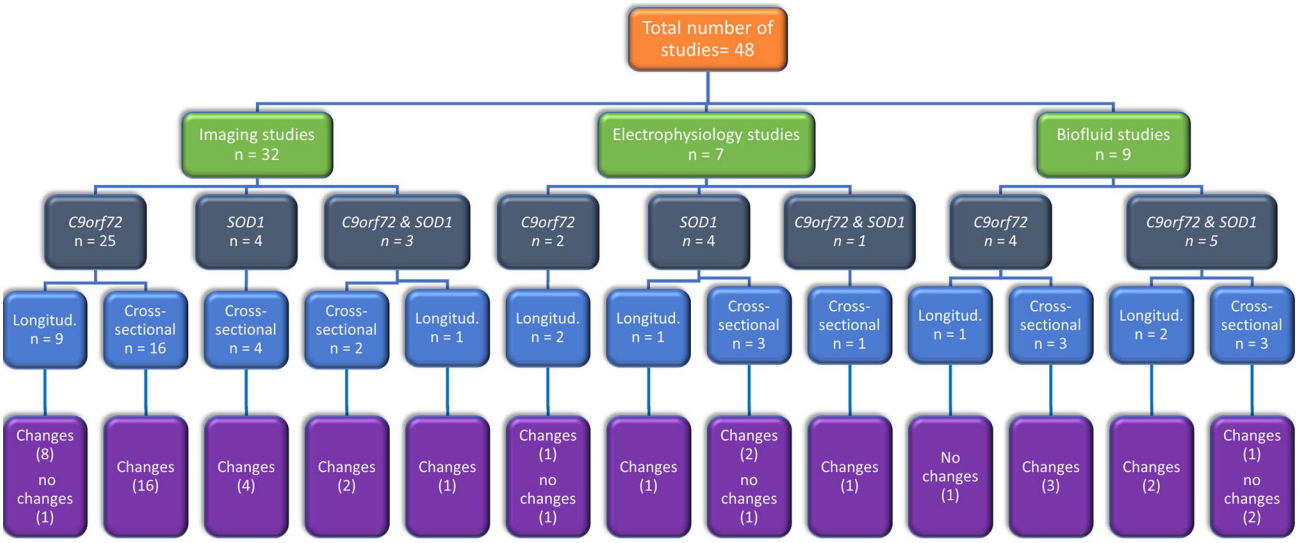

Fig. 1 Number of studies identified by the core search criteria

40, 41, 43–46] while others included sporadic patients [24, 32]. The size of the symptomatic cohort also shows great variation ranging from as little as 12 subjects [32] to 270 [31]. Symptomatic control cohorts included patients with ALS [10, 24, 28, 31, 35], FTD [24, 28] and ALS-FTD [24, 28]. Without exception, all identified studies included a cohort of healthy controls. These cohorts were either unrelated healthy controls [10, 24, 28, 29, 31–33, 35, 40], or more commonly, gene negative relatives of symptomatic patients [8, 23, 34, 36, 37, 42, 47]. A common shortcoming of the available papers is that when unrelated healthy controls were used as a reference group, their gene profile is seldom reported [30]. Furthermore, none of the reviewed studies used 'disease controls', which would have helped to gauge the specificity of findings to ALS. The mean age of asymptomatic *C9orf72* subjects ranged from 39.8 [7] to 51 years [29] and in the case of *SOD1*, carriers from 32.3 [34] to 47.2 years [10, 30]. In studies where a symptomatic cohort was included, their mean age ranged was 47.8 years [32] to 65.2 years [41]. Presymptomatic cohorts were generally relatives of symptomatic ALS [8, 27, 32], ALS-FTD or FTD patients [8, 27, 36] (Table 1).

Cohort size observations

Existing presymptomatic studies vary considerably with regards to overall sample size and statistical power. The total number of participants ranges from as few as 21 [34] to as many as 472 subjects [36]. While several studies included over 300 participants, these are invariably multi-centre studies necessitating some degree of inter-rater reliability testing for clinical assessments, sequence harmonisation for imaging and standard operating procedures for biomarker collection, storage and analysis [28, 31, 36, 40, 41]. Irrespective of the overall sample size, almost half all identified presymptomatic studies in ALS-FTD (15 out of 32) resulted from data generated from multi-site consortia such as the Genetic Frontotemporal dementia Initiative (GENFI) [28, 36, 38–46], the ARTFL–LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (ALLFTD) [37, 47] research consortium and the Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis Study Group (PREV-DEMALS) [7]. More than half of the identified studies (17 out of 32) were single-centre studies [10, 23–26, 32–35, 48]. Very few single-centre studies reached cohort sizes over 100 [49–51], and the largest single-centre recruited included 113 participants [50]. The most asymptomatic *C9orf72* participants included in a single-centre study were 40 participants [8] (Table 1).

Study methods

The vast majority, 22 out of the 32 presymptomatic imaging studies are cross-sectional [10, 33, 39, 45, 48], and only ten longitudinal presymptomatic studies can be presently identified [8, 24, 28, 30, 38, 43, 44, 47, 50, 51]. Significant variability can also be observed with regards to follow-up intervals, which range from 6 months [24] to 2 years [50, 51]. The longest overall follow-up period was 6 years [51]. The majority of longitudinal studies are 2 time-point studies, with a select few assessing subjects across three timepoints [24, 47, 51]. Only one study evaluated participants up to four times longitudinally [28]. T1-weighted structural MRI data were appraised in the majority (24) of presymptomatic imaging studies [28, 37, 38, 40–43, 45–47], diffusion MRI data in 14 studies [7, 8, 23, 27, 31, 33, 34] and functional MRI data in 6 studies

Table 1 A selection of presymptomatic neuroimaging studies in ALS/FTD

Authors, year of publication	Study participants	Clinical assessments	Imaging modality	Key findings in the presymptomatic cohort
Lulé et al. 2020 [30]	21 presymptomatic *C9orf72* 15 presymptomatic *SOD1* 91 healthy controls	HADS, ECAS, Verbal fluency, verbal and non-verbal memory, attention	Structural and diffusion MRI	- Reduced FA values in inferior frontal and orbitofrontal cerebral regions in C9orf72 repeat expansion carriers - Verbal fluency and non-verbal memory deficits identified in GGGGCC repeat carriers
de Vocht et al. 2020 [29]	17 presymptomatic *C9orf72* 25 healthy controls	Neurological exam, ECAS, MMSE, BDI	PET	-Hypometabolism in frontotemporal regions, insular cortices, basal ganglia, and thalami
Le Blanc et al. 2020 [28]	83 presymptomatic *C9orf72* 54 symptomatic carriers 249 healthy controls	MMSE, CBI	Structural MRI	-Reduced cortical thickness/cortical surface area in more restricted areas of medial frontoparietal lobes, in addition to scattered lateral frontal, parietal, and temporal areas
Staffaroni et al. 2020 [37]	127 presymptomatic 101 non carrier family members	Neurological and neuropsychological assessments, cognitive test—UDSNB-3, CDR	Structural MRI	-Individualized quantification of baseline brain atrophy is promising predictor of progression in asymptomatic familial FTD
Staffaroni et al. 2020 [47]	34 presymptomatic *C9orf72* 28 presymptomatic *GRN* 31 presymptomatic *MAPT* 78 non carrier family members	UDSNB, MOCA, TMT, CDR, NIH-EXAMINER	Structural MRI	-NIH-EXAMINER sensitive to cognitive changes and declines were associated with worsening clinical symptoms and brain volume loss
Cury et al. 2019 [45]	76 presymptomatic carriers 37 symptomatic carriers 98 non carrier family members	*All participants underwent GENFI assessments	Structural MRI	-Differences in shape in anterior region of thalamus at least five years before mutation carriers develop clinical symptoms
Feis et al. 2019 [49]	12 presymptomatic *C9orf72* 35 presymptomatic *GRN* 8 presymptomatic *MAPT* 48 non carrier family members	Neurological exam, neuropsychiatry tests, MMSE	Structural, diffusion and functional MRI	-Using newly created carrier-control classification model, mutation carriers can be separated from controls with a modest AUC even before symptom onset
Feis et al. 2019 [51]	12 presymptomatic *C9orf72* 35 presymptomatic *GRN* 8 presymptomatic *MAPT* 48 non carrier family members	Neurological exam, neuropsychiatry tests, MMSE	Structural, diffusion and functional MRI	-Mutation carriers did not have higher classification score increase over time than controls
Gazzina et al. 2019 [38]	31 presymptomatic *C9orf72* 65 presymptomatic *GRN* 20 presymptomatic *MAPT* 113 non carrier family members	*All participants underwent GENFI assessments MMSE scores reported in this study	Structural MRI	-Highly educated at-risk subjects had better cognition and higher GM volume at baseline -Higher educational attainment was associated with slower loss of GM over time

Table 1 (continued)

Authors, year of publication	Study participants	Clinical assessments	Imaging modality	Key findings in the presymptomatic cohort
Mutsaerts et al. 2019 [39]	34 presymptomatic *C9orf72* 55 presymptomatic *GRN* 18 presymptomatic *MAPT* 113 non carrier family members	*All participants underwent GENFI assessments MMSE, CBI-R and FDRS scores reported	Structural, arterial spin labelling	-CBF differences first appeared 12.5 years before expected symptom onset -CBF lower in mutation carriers closer to and beyond their expected age of symptom onset
Panman et al. 2019 [50]	12 presymptomatic *C9orf72* 33 presymptomatic *GRN* 15 presymptomatic *MAPT* 53 non carrier family members	Neurological exam, neuropsychological assessment, MMSE, NPI-Q	Structural and diffusion MRI	-Lower GM volume in cerebellum and insula -WM differences in anterior thalamic radiation, at baseline and follow-up
Premi et al. 2019 [36]	82 presymptomatic *C9orf72* 122 presymptomatic *GRN* 45 presymptomatic *MAPT* 223 non carrier family members	*All participants underwent GENFI assessments TMT, MMSE, CBI scores reported in this study	Functional MRI	-Diminished dynamic fluidity -Visiting less meta-states -Shifting less often across them -Travelling through a narrowed meta-state distance
Querin et al. 2019 [8]	40 presymptomatic *C9orf72* 32 non carrier family members	UMN and LMN signs, bulbar signs, cognitive and behavioural changes, neuropsychological exam, ALSFRS-R, MMSE, FAB, MDRS	Diffusion MRI	-Significant CST FA reduction at baseline
Rittman et al. 2019 [44]	17 presymptomatic *C9orf72* 13 presymptomatic *MAPT* 38 presymptomatic *GRN* 24 symptomatic carriers 80 non carrier family members	*All participants underwent GENFI assessments Scores not reported in this study	Functional MRI	-Despite loss of both brain volume and functional connections, there is maintenance of efficient topological organization of brain's functional network in years leading up to estimated age of FTD symptom onset
Tavares et al. 2019 [43]	13 presymptomatic *C9orf72* 29 presymptomatic *GRN* 4 presymptomatic *MAPT* 18 symptomatic carriers 56 non carrier family members	*All participants underwent GENFI assessments Scores not reported in this study	Structural MRI	-Ventricular volume differences are detectable
Wen et al. 2019 [27]	38 presymptomatic *C9orf72* 29 non carrier family members	Neurological exam, neuropsychological and behavioural assessments, MMSE, FAB, MDRS	Structural and diffusion (NODDI) MRI	-WM abnormalities in ten tracts with neurite density index and only five tracts with DTI metrics
Bertrand et al. 2018 [7]	41 presymptomatic *C9orf72* 39 non carrier family members	Neurological exam, neuropsychological and behavioural scores, MMSE, MDRS, FBI, FAB, SEA, Praxis score, Benson figure, Free and cued recall test, Fluency tasks	Structural, diffusion MRI	-Atrophy in four cortical regions of interest and in right thalamus -WM alterations in two tracts in young *C9orf72*-positive individuals
Cash et al. 2018 [41]	40 presymptomatic *C9orf72* 65 presymptomatic *GRN* 23 presymptomatic *MAPT* 47 symptomatic carriers 144 non carrier family members	*All participants underwent GENFI assessments MMSE scores reported in this study	Structural MRI	-GM atrophy in the thalamus and cerebellum

Table 1 (continued)

Authors, year of publication	Study participants	Clinical assessments	Imaging modality	Key findings in the presymptomatic cohort
Fumagalli et al. 2018 [40]	42 presymptomatic *C9orf72* 66 presymptomatic *GRN* 24 presymptomatic *MAPT* 63 symptomatic carriers 148 non carrier family members	*All participants underwent GENFI assessments	Structural MRI	-Widespread atrophy in *C9orf72*
Popuri et al. 2018 [48]	15 presymptomatic *C9orf72* 9 presymptomatic *GRN* 38 non carrier family members	Neurological exam including motor and reflexes, FAB, FBI, MMSE	Structural MRI	-Cortical thinning in temporal, parietal and frontal regions -Reduced volumes of bilateral thalamus and left caudate
Gorges et al. 2017 [31]	18 presymptomatic *C9orf72* 11 presymptomatic *SOD1* 1 presymptomatic *NEK1* 1 presymptomatic *TARDBP* 1 presymptomatic *FUS* 270 symptomatic patients 112 healthy controls	Neurological exam, ALSFRS-R	Structural and diffusion MRI	-Atrophy of the hypothalamus
Papma et al. 2017 [26]	18 presymptomatic *C9orf72* 15 non carrier family members	Neuropsychiatric tests	Structural and diffusion MRI	-WM integrity loss in tracts connecting frontal lobe, thalamic radiation, and tracts associated with motor functioning -GM volume loss in thalamus, cerebellum, and parietal and temporal cortex in C9orf72 positive above 40 years of age
Premi et al. 2017 [42]	33 presymptomatic *C9orf72* 14 presymptomatic *MAPT* 61 presymptomatic *GRN* 123 non carrier family members	*All participants underwent GENFI assessments	Structural MRI	-Lower GM volume
Sudre et al. 2017 [46]	28 presymptomatic *C9orf72* 25 presymptomatic *GRN* 8 presymptomatic *MAPT* 43 symptomatic carriers 76 non carrier family members	*All participants underwent GENFI assessments Scores not reported in this study	Structural MRI	-Significant differences in symptomatic *GRN* group -No differences in the *MAPT* or *C9orf72* groups
Floeter et al. 2016 [24]	7 asymptomatic *C9orf72* 42 symptomatic carriers/sporadic 28 healthy controls	Neurological exam, ALSFRS-R, D-KEFS, MMSE, MDRS	Structural MRI	-Study unable to identify structural changes in asymptomatic carriers
Lee et al. 2016 [25]	15 presymptomatic *C9orf72* 67 non carrier family members	Neurological exam-UMN and LMN signs, cognitive testing, MMSE, CVLT, CDR, neuropsychology	Structural, diffusion and functional MRI	-GM volume deficits in cingulate, insula, thalamus, and striatum -Reduced WM integrity found in corpus callosum, cingulum bundles, corticospinal tracts, uncinate fasciculi and inferior longitudinal fasciculi

Table 1 (continued)

Authors, year of publication	Study participants	Clinical assessments	Imaging modality	Key findings in the presymptomatic cohort
Menke et al. 2016 [32]	2 presymptomatic *C9orf72* 10 presymptomatic *SOD1* 12 symptomatic sporadic patients 12 healthy controls	Neuromuscular exam, neuropsychological test, ALSFRS-R	Structural, diffusion and functional MRI	-Widespread FA and RD differences - FC between cerebellum and network comprising precuneus, cingulate and middle frontal lobe significantly higher
Walhout et al. 2015 [23]	16 presymptomatic *C9orf72* 14 symptomatic carriers 51 non carrier family members	NA	Structural and diffusion MRI	-Cortical thinning in temporal, parietal, and occipital - Left caudate and putamen atrophy
Carew et al. 2011 [10]	24 presymptomatic *SOD1* 23 symptomatic carriers 29 healthy controls	Neurological exam-UMN and LMN signs, ALSFRS-R, FVC	MRS	- Reduced NAA/Cr and NAA/Myo ratios in the superior spinal cord
Vucic et al. 2010 [33]	7 presymptomatic *SOD1* 62 healthy controls	MRC, ALSFRS-R, Triggs hand function score	Diffusion MRI and TMS	-Combined anatomical and functional modalities established normal integrity of corticomotoneurons
Ng et al. 2008 [34]	8 presymptomatic *SOD1* 13 non carrier family members	Neurological exam, neuropsychology	Structural and diffusion MRI	-Decreased FA and increased tensor trace (TT) at posterior limb of internal capsule - Increased RD was detected on both sides
Turner et al. 2005 [35]	2 *SOD1* 34 symptomatic carriers 24 healthy controls	Neurological exam, ALSFRS-R, UMN-score	PET	-Small focus of reduced [11C]flumazenil binding at left fronto-temporal junction similar to the pattern seen in the clinically affected patients

*GENFI assessments include physical examination, FDR, CBI-R, Uniform data set: Weschler memory scale revised, digit symbol, TMT, BNT, category fluency, letter fluency, Wechsler Abbreviated Scale of Intelligence Block Design, MMSE [52]

[25, 32, 36, 44, 49, 51]. MR spectroscopy was performed in one study [10] and one study used arterial spin labelling [39]. Two presymptomatic PET studies were identified [29, 35], one using flumazenil [35], and the other used fluorodeoxyglucose (F-FDG) [29] as a tracer. While many studies investigated a single parameter, 14 studies implemented a multi-modal approach [7, 23–26, 29, 32–34, 39, 49–51]. The majority of MRI studies were performed on a 3 T MRI platform [7, 8, 10, 28, 33]. Seven studies relied on imaging data from a 1.5 T scanner [34] and of these, 5 were multi-site studies relying on mixed data from 1.5 T and 3 T scanners [31, 38, 40, 41, 44]. No ultra-high field (7 T) human presymptomatic studies were identified at the time of this review (Table 1).

Imaging findings in presymptomatic ALS

In *C9orf72* hexanucleotide repeat expansion carriers, frontal [28], temporal, parietal, occipital [23], thalamic [25, 26], cerebellar [26] and striatal [25] atrophy was consistently detected. Diffusion MRI captured reduced WM integrity in the corticospinal tracts [8, 25], orbitofrontal regions [30], corpus callosum, cingulum, uncinate and inferior longitudinal fasciculi [7, 25, 27]. Neurite orientation dispersion and density imaging (NODDI) is thought to be more sensitive in detecting white matter alterations than traditional DTI metrics [27]. A PET study of presymptomatic *C9orf72* participants confirmed hypometabolism in frontotemporal, insular, thalamic and basal ganglia regions [29]. It is noteworthy, that some studies did not detect cerebral changes in asymptomatic *C9orf72* carriers [24].

In asymptomatic *SOD1* carriers, white matter degeneration was observed in the posterior limb of the internal capsule [34], reduced flumazenil binding in the left frontotemporal junction [35], and reduced NAA/Cr and NAA/Myo ratios in the superior spinal cord [10]. A multimodal study of seven asymptomatic SOD1 carriers found no significant abnormalities on diffusion tensor imaging and threshold tracking transcranial magnetic stimulation [33].

In asymptomatic mixed-genotype cohorts frontal, temporal, parietal [48] and cerebellar [41] atrophy was noted as well as subcortical grey matter degeneration including the caudate [48], hypothalamus [31], and thalamus [41, 48]. White matter alterations were observed in the anterior thalamic radiation [50]. Marked connectivity changes were detected by some functional MRI studies [32, 36], while others identified functional resilience despite structural degeneration [44] (Table 1).

The clinical profile of mutation carriers

Accompanying clinical assessments

Most presymptomatic imaging studies incorporate a brief neurological assessment to screen for clinical signs [24, 27, 29, 31, 32, 34, 35, 37, 49–51], but the details of the exam are seldom reported [8, 10, 25, 48]. In symptomatic patients, the revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-r) [8, 10, 24, 31–33, 35], Medical Research Council (MRC) scale [33], Trigg's hand function score [33], and composite upper motor neuron (UMN) scores [35] are typically administered. Nineteen out of 32 studies also commented on neuropsychological performance. The battery of neuropsychological instruments varied greatly across the identified studies. Many studies used generic, non-ALS specific, screening tests such as the Mini Mental State Examination (MMSE) [7, 8, 24, 25, 27–29, 36, 38, 41, 42, 48–51], the Montreal Cognitive Assessment (MOCA) [47], the Frontal Assessment Battery (FAB) [7, 27, 48], the Clinical Dementia Rating Scale (CDR) [47], or the Mattis Dementia rating scale (MDRS). Some centres relied on ALS-specific screening tools such as the (ECAS) [25, 29, 30], while others used an extensive battery of neuropsychological tests assessing memory, visuospatial, language, executive domains, anxiety and depression [25]. Some studies focused on specific cognitive domains known to be preferentially affected in ALS, such as executive function. This was typically interrogated by digit span [42], Stroop test [26], trail making test (TMT) [36, 42, 47], fluency tasks [7, 26, 42], the Delis–Kaplan Executive Function System (D-KEFS) [24] or symbol digit modalities test (SDMT) [42]. Language was either assessed by the Boston naming test [7, 42] or the Wide Range Achievement Test (WRAT) [25]. Memory performance was appraised using the California Verbal Learning test (CVLT) and Benson figure recall [25] and other recall tests [7, 42]. Visuospatial function was assessed using the Benson figure and The Visual Object and Space Perception Battery (VOSP) [25]. Possible neuropsychiatric manifestations have been assessed by the Neuropsychiatric Inventory Questionnaire (NPI-Q) [25, 50], and depression has been screened for by the geriatric depression scale [25] or the Beck Depression Inventory (BDI) [25, 29]. While deficits in social cognition are recognised in symptomatic ALS, these are seldom assessed specifically in presymptomatic cohorts [30, 53–55]. Presymptomatic behavioural manifestations were evaluated by the revised Cambridge Behavioural Inventory (CBI-R), [28, 36, 42] and the Frontal Behavioural Inventory (FBI) [7, 24, 48]. ALS-specific behavioural instruments [56, 57] were not applied to presymptomatic cohorts. Other instruments used in presymptomatic studies included the neuropsychological battery of the Uniform Data Set (UDSNB) and the Executive Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research (EXAMINER) [47].

Clinical findings in presymptomatic cohorts

A study of presymptomatic *C9orf72* carriers identified subtle deficits in executive functioning, verbal fluency [30]

and memory [29], while another study detected significant memory impairment [25]. Several studies found that MMSE scores are slightly lower in presymptomatic *C9orf72* carriers, but still within normal range [7, 25, 28, 29, 48]. Performance on other tests such as the MDRS [7], FAB [7, 48], FBI [7, 48], Benson figure [7, 25], Boston naming test [7, 25], Stroop, verbal fluency, and digit span [25] is thought to be relatively preserved and comparable to healthy controls.

Electrophysiology studies of asymptomatic mutation carriers

Presymptomatic mutation carriers at risk of developing ALS have also been extensively investigated by quantitative electrophysiology tools (Table 2), such as motor unit number estimation (MUNE) [21, 22, 58], magnetoencephalography (MEG) [59], and transcranial magnetic stimulation (TMS) [33, 60–62]. Some of these studies also interrogated accompanying MRI data [33, 59, 62]. Asymptomatic *C9orf72* [59, 60, 62] and *SOD1* [21, 22, 33, 58, 59, 61] mutation carriers are typically either compared to unrelated healthy controls [33, 59–61], gene-negative family members [62] or both [21, 22, 58]. Longitudinal studies [21, 60, 62] followed patients for up to 3 years [21, 60] with a follow-up interval as short as 6 months [21]. The number of total participants in presymptomatic electrophysiology studies range from 52 [59] to 186 [62] with up to 19 presymptomatic *SOD1* [21, 58] and up to 11 presymptomatic *C9orf72* [60] carriers included in any one study. The majority of these studies also include a group of symptomatic patients [21, 22, 59–61], and often symptomatic mutation carriers [62]. Symptomatic patients were mostly ALS [21, 22, 59–61] or FTD patients [62], but one study also included PLS patients [59]. None of the reviewed studies included neurodegenerative 'disease-controls'. The age profile of presymptomatic mutation carriers in electrophysiology studies range from 40 [61] to 51.7 [59] but several studies did not report demographic data in detail. In presymptomatic electrophysiology studies, the presymptomatic cohort was on average 10 years younger than the symptomatic cohort. On TMS, alterations in intracortical facilitation transmission are seen up to 3 decades before expected symptom onset [62]. One study showed that SICI was absent in only two presymptomatic *SOD1* carriers and reduced in one [61]. A study investigating MUNE showed no changes in presymptomatic *SOD1* carriers [22], but a follow-up study reported that 2 of 19 *SOD1* carriers showed reduction in MUNE just months before the symptom onset [21]. Cortical hyperexcitability was detected in symptomatic *C9orf72* carriers but not asymptomatic carriers [60]. Some electrophysiology studies report the clinical profile, including MRC scores in presymptomatic cohorts [21, 58, 61] or ALSFRS-r in symptomatic patients [59–61]. Cognitive screening with ECAS [59] or MMSE [62] was also implemented in some studies.

Biofluid studies of asymptomatic mutation carriers

In the era of 'omics' (proteomics, lipidomics, metabolomics etc.) biofluid markers are also increasingly evaluated in asymptomatic mutation carriers including neurofilament light (NfL) [63–65] or (NEFL) [66], neurofilament heavy (pNfH) [64, 65, 67] or NEFH [66], poly(GP) proteins [68, 69], chitotriosidase-1 (CHIT1) [66, 67, 70], chitinase 3-like protein 1 (CHI3L1) [66, 67], chitinase 3-like protein 2 (CHI3L2) [67], C-reactive protein (CRP) [67], mitochondrial DNA (mtDNA) [71], ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) [66, 70], microtubule-associated protein 2 (MAP2) [66], macrophage-capping protein (CAPG) [66], glycoprotein non-metastatic B (GPNMB) [66], histone cluster 1, H4 (HIST1H4A) [66], histone cluster 1, H2b (HIST1H2B) [66], neurofilament medium (NEFM) [66, 70], neuronal pentraxin receptor (NPTXR) [70]. Some studies focused on protein profiles in a single biofluid either in the CSF [66, 67, 69, 70] or serum [71], while others evalauted both CSF and serum [63–65, 68]. The most commonly used methods to quantify the concentration of these markers are enzyme-linked immunosorbent assays (ELISA) [64, 65, 67], electrochemiluminescence immunoassay (ECLIA) [63, 65], mass spectroscopy [66, 70], meso scale discovery-based immunoassay [68] and poly-GP immunoassay [69]. Some longitudinal studies, followed asymptomatic mutation carriers for over 3 years [63, 64], and in some studies controls were also assessed longitudinally [63, 64]. Most biofluid studies investigated asymptomatic *SOD1* [63–66, 71] and *C9orf72* [63–71] cohorts, but some included *TARDBP* [65, 66] or *FUS* [65] mutation carriers. The biggest biofluid study included 84 subjects, including 52 *SOD1* and 27 *C9orf72* hexanucleotide carriers among other mutation carriers [63]. All identified studies also concurrently assessed a symptomatic cohort of either ALS [63–70], ALS-FTD [68] or FTD [69] patients. Some studies included a PLS cohort [67, 68], as well as disease controls such as Alzheimer's disease [68, 69], Lewy body dementia [68], Parkinson's disease [69], or Kennedy's disease [67]. Mean age of asymptomatic mutation carriers ranged from 39.7 [67] to 48.3 years [65]. Some biofluid studies screened for the presence of UMN and LMN signs [65]. In symptomatic patients, ALSFRS-r was invariably recorded, and some studies also reported UMN [64, 67], ECAS [64, 67] or FTD-CDR scores [69]. Presymptomatic neurofilament studies are inconsistent; many did not detect elevated levels [65–67]. Altered protein profiles were identified by some studies [68–70], especially in the years preceding phenoconversion [63, 64] (Table 3).

Table 2 A selection of presymptomatic electrophysiology studies in ALS

Authors and year of publication	Study participants	Clinical assessments	Methods	Significant findings in presymptomatic cohort
Benussi et al. 2019 [62]	4 presymptomatic *C9orf72* 48 presymptomatic *GRN* 61 symptomatic carriers 73 non carrier family members	Neuropsychological assessment, CBI-R, MMSE, TMT	TMS, structural MRI	-Biological changes and intracortical facilitation transmission abnormalities occur 3 decades before, followed by intracortical inhibition transmission deficits 2 decades before expected symptom onset, followed by an increase of WM lesions, brain atrophy, and cognitive impairment
Proudfoot et al. 2017 [59]	10 presymptomatic *SOD1* 2 presymptomatic *C9orf72* 20 symptomatic carriers/sporadic 20 healthy controls	Cognitive tests, ALSFRS-R, ECAS, ACE-R	MEG, structural MRI	-Movement execution coincided with excess beta desynchronization
Geevasinga et al. 2015 [60]	11 presymptomatic *C9orf72* 88 symptomatic carriers/sporadic 74 healthy controls	ALSFRS-R, MRC, UMN score	TMS-MEP, CMAP	-Cortical hyperexcitability is an intrinsic feature of symptomatic c9orf72 but not asymptomatic
Aggarwal et al. 2012 [58]	19 presymptomatic *SOD1* 34 non carrier family members 16 healthy controls	Neurological exam, MRC	MUNE	-MUNE is more sensitive for monitoring disease progression than maximal voluntary isometric contraction (MVIC), as MUNE correlates with number of functional motor neurones
Vucic et al. 2008 [61]	17 presymptomatic *SOD1* 57 symptomatic carriers/sporadic 55 healthy controls	Neurological exam, neuropsychological test, ALSFRS-R, MRC, Triggs hand function score	TMS	- SICI was completely absent in 2 pre-symptomatic SOD-1, in 1 subject there was a 32% reduction in SICI -These three individuals subsequently developed clinical features of ALS
Aggarwal et al. 2002 [21]	19 presymptomatic *SOD1* 12 symptomatic sporadic 34 non carrier family members 23 healthy controls	Neurological exam, MRC	MUNE	-In 2 of 19 mutation carriers, there was a sudden reduction in MUNE several months before the onset of weakness
Aggarwal et al. 2001 [22]	18 presymptomatic *SOD1* 12 symptomatic sporadic 34 non carrier family members 23 healthy controls	N/a	MUNE	-No detectable difference in the number of motor units

Lessons from other neurodegenerative conditions

Despite the clinical differences, frontotemporal dementia studies offer ample learning opportunities for ALS study designs. In addition *C9orf72*, other FTD-associated mutations have been extensively investigated, including *GRN* [72–84], *MAPT* [75, 85–88] and CHMP2B [89–91] carriers. Mutation carriers were more commonly compared to gene-negative first-degree relatives [72, 75, 76, 78–80, 88–91], but also to healthy controls [73, 74, 77, 81, 86, 87] or both [82, 84]. The average age of presymptomatic cohorts in FTD ranges from 31 [88] to 56 years [91]. Many of the reviewed studies also investigated a symptomatic cohort, but a few only focussed on their presymptomatic cohort [74–76, 84, 87, 89–91]. Similarly to ALS, no studies included 'disease controls'. Neuropsychological data was more commonly included than in ALS studies, including screening tests such as the CDR [72, 79–81], MOCA [72] and MMSE [73, 74, 78, 82, 87], behavioural tools such as the FBI [78] and FAB [78], neuropsychiatric instruments such as the NPI [78, 82] or BDI [87], as well as executive [73–75, 82, 87], language [73, 74, 87], visuospatial [73, 82] and memory tests [73,

Table 3 A selection of presymptomatic biofluid studies

Authors	Proteins investigated	Study participants	Clinical tests	Methods	Findings in presymptomatic subjects
Barshcke et al. 2020 [70]	2095 proteins identified, eight candidates validated- UCHL1, NPTXR, etc	Discovery- 11 presymptomatic *C9orf72* MRM validation- 28 presymptomatic *C9orf72* SIMOA validation- 13 presymptomatic *C9orf72* 27 controls- either healthy controls/non carrier family members	ALSFRS-R, FTLD-CDR	iTRAQ, mass spectroscopy- Multiple reaction monitoring (MRM)	-Decreased neuronal pentraxin receptor (NPTXR) levels in *C9orf72*-FTD versus carriers
Oeckl et al. 2020 [66]	UCHL1, MAP2, CAPG, GPNMB, HIST1H4A, HIST1H2B, NEFL, NEFH, NEFM, CHIT1, CHI3L1	Discovery: 8 presymptomatic *C9orf72* 5 presymptomatic *SOD1* 1 presymptomatic *TARDBP* 26 symptomatic carriers/ sporadic 16 non carrier family members Validation 85 symptomatic carriers/ sporadic 32 healthy controls	ALSFRS-R	iTRAQ, mass spectroscopy- MRM	-No significant alteration observed in asymptomatic mutation carriers
Benatar et al. 2019 [64]	Neurofilament heavy (pNfH), neurofilament light (NfL)	25 presymptomatic *C9orf72* 49 presymptomatic *SOD1* 5 other presymptomatic gene carriers 36 symptomatic carriers/ sporadic 34 non carrier family members	ALSFRS-R, UMN burden score, ECAS	ELISA	-Serum and CSF pNfH increase prior to phenoconversion -changes observed 6–12 months in *SOD1*, 2 years in *C9orf72*, and 3.5 years in *FUS* prior to disease onset
Thompson et al. 2019 [67]	CHIT1, CHI3L1, CHI3L2, pNFH, CRP	5 Asymptomatic *C9orf72* 92 symptomatic sporadic 12 ALS-mimics 25 healthy controls	ALSFRS-R, UMN burden score, ECAS	ELISA	-Chitinase levels were similar in asymptomatic mutation carriers and healthy controls
Benatar et al. 2018 [63]	Neurofilament light (NfL)	52 presymptomatic *SOD1* 27 presymptomatic *C9orf72* 5 other presymptomatic 34 non carrier family members	ALSFRS-R	ECLIA	Aamong phenoconverters, NfL levels were raised ~12 months preceding the emergence of the earliest clinical symptoms
Stoccoro et al. 2018 [71]	mtDNA	N/A	N/A	N/A	-Significant decrease in D-loop methylation levels

Table 3 (continued)

Authors	Proteins investigated	Study participants	Clinical tests	Methods	Findings in presymptomatic subjects
Gendron et al. 2017 [68]	poly(GP) proteins	27 presymptomatic *C9orf72* 107 symptomatic *C9orf72* carriers 48 gene-negative healthy controls 57 gene-negative ALS patients	ALSFRS-R	Meso Scale Discovery-based immunoassay	-Poly(GP) proteins detected in CSF and in peripheral blood mononuclear cells
Lehmer et al. 2017 [69]	poly(GP) proteins	10 presymptomatic *C9orf72* 49 symptomatic carriers/ sporadic 20 healthy controls 8 non carrier family members 38 disease controls	Neuropsychological testing, ALSFRS-R, FTLD-CDR	poly-GP immunoassay	-Significant poly-GP levels detectable similar to symptomatic carriers
Weydt et al. 2016 [65]	Neurofilament heavy (pNfH), neurofilament light (NfL)	7 presymptomatic *C9orf72* 3 presymptomatic *SOD1* 1 presymptomatic *TARDBP* 1 presymptomatic *FUS* 64 symptomatic carriers 19 non carrier family members	Neurological exam- UMN and LMN signs, ALSFRS-R	ELISA, ECLIA	-NF-L and pNF-H are normal before symptom onset

82, 87, 88]. While a minority of studies investigated a single imaging parameter [72, 80, 89], most studies presented structural data as well as diffusion [74, 75, 84, 86], functional [73, 75, 77, 79, 81, 82] or PET data [76, 78, 85, 91]. Most of the longitudinal studies were two time-point studies [72, 74, 76, 87, 89–91], but one multi-timepoint study followed patients over 11 years [88]. Robust multi-centre initiatives such as Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects (LEFFTDS) [72, 88] and The Genetic Frontotemporal dementia Initiative (GENFI) have been particularly successful at gathering large datasets. One of the most widely studied neurodegenerative condition in its presymptomatic phase is Huntington's disease which has been extensively evaluated by structural [92–98], diffusion [95, 99] and functional [97, 100] MRI studies. These studies tend to be much larger than ALS studies and often include data from hundreds of participants [92, 94]. Multi-centre initiatives such as PREDICT-HD [92, 94], IMAGE-HD [93, 98] and TRACK-On HD [95, 97] have facilitated these robust collaborative studies. In presymptomatic Alzheimer's studies, APP [101–105], PSEN1 [101–107], PSEN2 [101–105] and APOE4 [101, 104, 105, 108–110] mutation carriers were investigated with some studies including over 300 participants [102, 103]. In presymptomatic Parkinson's disease, LRRK2 [111–118], parkin [114, 118–120], PINK1 [118], ATP13A2 [118] and SNCA [121] mutation carriers were evaluated. These studies are relatively smaller than those conducted in HD and AD, but can include as much as 130 participants [118].

Discussion

Irrespective of their methodology and genetic focus, the majority of existing presymptomatic ALS studies have confirmed considerable biological changes before symptom manifestation. They are also consistent in identifying changes in brain regions which are characteristically affected in symptomatic mutation carriers. The demographic analysis of existing *C9orf72* studies highlights that mutation carriers in their 30 s already exhibit considerable structural degeneration, decades before typical symptom manifestation [7, 8]. The considerable pathological changes detected in young mutation carriers raises questions about neurodevelopmental factors [6, 30, 122], but this could only be appraised if mutation carriers in their teens and twenties were also included subject to appropriate approvals and genetic counselling. Existing studies suggest a relatively divergent imaging signature in asymptomatic *C9orf72* and *SOD1* carriers, but in the absence of well-powered studies including large cohorts of both mutations, these genotype-specific traits are not firmly established. Nonetheless, the presymptomatic signature of *C9orf72* seems to be associated with more

widespread frontotemporal and subcortical grey matter degeneration than those observed in association with SOD1 [7, 27]. While the available studies are conceptually important, stereotypical shortcomings can be readily identified. The sample size of asymptomatic mutation carriers in ALS imaging studies range from 2 to 83, which coupled with the considerable variation in the age of participants, prevents making conclusive observations regarding presymptomatic biology. The sample size limitations of presymptomatic ALS studies are particularly striking in contrast to the available AD and HD literature. The systematic review of the literature also highlights that in contrast to presymptomatic AD and HD studies, the majority of presymptomatic studies in ALS are single-centre, or national studies. Several ALS studies have included both *SOD1* and *C9orf72* carriers in a single presymptomatic group to demonstrate structural changes before symptom manifestation. However, with the emergence of ASO therapies, it seems paramount to describe genotype-specific changes in a specific presymptomatic mutation cohort rather than characterising admixed cohorts. The importance of defining genetically homogenous groups cannot be underestimated, as carriers of specific mutation may exhibit different rate of progression and anatomical involvement. The strategy to ascertain presymptomatic changes also varies considerably in ALS studies; some research groups contrast their presymptomatic cohort to healthy controls alone, others to symptomatic patients, and others to gene-negative family members. Another common problem is the selection of symptomatic patients. Including a symptomatic patient cohort in presymptomatic studies is particularly useful if they carry the same mutation as the presymptomatic cohort, but the inclusion of a mixed gene-positive and gene-negative sporadic patients, or symptomatic patients without genetic profiling hinders data interpretability. If the symptomatic cohort carries the same mutation, their "affected" brain regions can be specifically evaluated in the presymptomatic group in targeted region-of-interest analyses. Another potential shortcoming of existing studies is the lack of disease controls which makes it difficult to appraise how specific the findings are to ALS or to a given genotype. For example, corpus callosum degeneration is regarded as pathognomonic of ALS by the ALS research community, despite being observed in a range of other conditions such as HSP to AD. Similarly, increased neurofilament levels were observed in a number of presymptomatic ALS studies, but they are also raised in many other neurological conditions. If no disease-controls are included, the specificity of a biomarker to ALS is impossible to assess and its ability to distinguish between neurodegenerative processes remain questionable. For example, if a proposed biomarker such as CSF neurofilaments, corticospinal tract FA or a TMS parameter is similarly affected in ALS, PLS and CBD, it will not distinguish between these conditions rendering their diagnostic value relatively limited. In real-life clinical scenarios, the question is seldom whether a patient is healthy or not, the question is typically whether the constellation of symptoms presage ALS or PLS or CBD, as these conditions carry distinctly different prognoses. Not only existing studies do not include disease controls, they do not include other motor neuron disease phenotypes either. This seems like a missed opportunity. The inclusion of relatively pure UMN and LMN phenotypes, such as adult SMA or PLS may help to gauge the sensitivity of a proposed marker to the UMN versus LMN system [123–125]. Another contentious aspect of existing studies is the generalisation of observations from small presymptomatic *C9orf72* and *SOD1* cohorts as representative of presymptomatic ALS as a whole. Depending on the population, the vast majority of ALS patients test negative for ALS-associated mutations and are seemingly sporadic. Accordingly, the presymptomatic phase of sporadic patients remains a conundrum and may differ in anatomical involvement and chronological dynamics from the traits observed in *C9orf72* or *SOD1*. This also applies to PLS, which is not closely linked to single mutations and very little is known about cerebral and spinal disease burden prior to symptom onset. PLS exhibits overlapping albeit UMN predominant clinical and radiological characteristics with ALS [123], and in the absence of specific mutations to carry out presymptomatic studies, research groups attempted to characterise 'early' symptomatic cohorts before fulfilling diagnostic criteria [124, 126]. These therefore cannot be regarded as presymptomatic studies, rather pre-diagnosis studies of suspected cohorts. The lessons of these studies can be integrated in future ALS studies, namely that gene negative 'suspected ALS' patients should also be included in imaging and biomarker studies in an attempt to characterise early pathology in sporadic cohorts. Additionally, presymptomatic gene-positive cohorts should be followed beyond symptom manifestation and at least until they fulfil current diagnostic criteria for ALS. Characterising disease burden by quantitative imaging, electrophysiology and wet biomarker protocols at the time of fulfilling diagnostic criteria, may help to highlight the limitations of existing diagnostic criteria. The refinement of diagnostic criteria may enable an earlier diagnosis in suspected patients and in turn earlier inclusion in clinical trials. One aspiration would be the introduction of 'radiologically-supported ALS' based on objective radiological variables. Another important question is the optimal timing ASO therapy. Once brain and cord pathology is detected and electrophysiology changes ascertained in mutation carriers there may be an argument to introduce therapy early before widespread irreversible changes ensue. Existing presymptomatic studies also raise important question re: motor reserve. The observation that both electrophysiology and MRI detects considerable pyramidal tract, motor cortex and spinal cord degeneration long before

projected symptom manifestation suggest a degree of network resilience or redundancy. A simplistic interpretation may be that compensatory processes and redundant networks offset degenerative changes until a critical threshold is reached.

While cross-sectional studies have provided pioneering insights, they provide limited information on progressive changes as they average radiological signatures across different age groups. With few exceptions [7], existing presymptomatic studies in ALS rely on convenience samples of asymptomatic mutation carriers with considerable dispersion in their demographic profile. For the meaningful analysis of early alterations, demographically homogenous samples would be desirable. Longitudinal studies have the potential to provide a nuanced picture of progressive changes; map anatomical propagation patterns, progressive functional alterations and evolving CSF/serum signatures overtime. However these studies should also ideally recruit demographically homogenous cohorts. The limitations of two timepoint longitudinal designs are also clear, as these don't permit the modelling of non-linear changes and the assessment of ceiling- and flooring-effects [127]. The lessons of large AD and HD studies also apply here, namely that large multi-timepoint designs are necessary to characterise progressive structural degeneration in ALS. A limitation of existing longitudinal presymptomatic studies in ALS is that mutation carriers are seldom followed until phenoconversion or beyond. The availability of radiological, electrophysiology or wet biomarker panel in mutation carriers before and after symptom manifestation would also permit the evaluation of prognostic indicators. There are two practical deliverables which were not addressed by existing studies, both of which seem relevant for individualised patient care and future pharmacological trials. One of them is the estimation of projected age of symptom onset based on disease burden in the presymptomatic phase. This would be possible if mutation carriers would be meticulously followed until symptom manifestation. The other practical aspect of presymptomatic studies pertains to *C9orf72* from a phenotype point-of-view; namely can patterns of cerebral or spinal cord involvement be used to predict if an individual GGGGCC repeat expansion carrier is more likely to develop FTD or ALS (ALS-FTD). Clinical and radiological data have been previously used to build prognostic models for individual symptomatic patients, but these are yet to be applied to presymptomatic individuals [128–131]. These observations highlight another shortcoming of existing presymptomatic studies in ALS; with very few exceptions [8, 10] nearly all presymptomatic radiology studies are brain studies. Spinal cord involvement is a key aspect of ALS, which encompasses anterior horn (LMN) and descending pyramidal tract (UMN) degeneration and is now readily detected by novel imaging applications [132, 133]. Given the availability of robust quantitative spinal protocols, these should be carefully integrated into future presymptomatic studies to assess if they detect changes earlier than brain protocols and if they can presage age of onset, site of onset, or UMN/LMN predominance. It is conceivable that a hexanucleotide carrier with ample extra-motor cerebral involvement with no spinal cord abnormalities is more likely to develop FTD, than ALS, but unless such studies are conducted and patients followed until disease manifestations the predictive value of presymptomatic imaging is difficult to gauge. The practical deliverables of robust presymptomatic studies therefore include phenotypic prediction, age of onset estimation and optimising the timing of pharmacological interventions Table 4. Very few presymptomatic studies report negative or unexpected findings [33, 60]. The candid reporting of negative results is hugely important as they either reveal genotype-specific traits or reflect on the detection sensitivity of the methods implemented. Similarly, the comparative evaluation of several imaging metrics in the same cohort is helpful to appraise the detection sensitivity of specific methods. Some pioneering spectroscopy studies for example did not perform accompanying structural assessments and vice versa [10]. With the current imaging technology at our disposal the detection of white and grey matter alterations in symptomatic ALS cohorts is no longer challenging [134, 135], but the concomitant implementation of several imaging modalities in the presymptomatic phase enables the critical comparison of various techniques. For example NODDI is thought to be superior to characterise white matter degeneration in presymptomatic cohorts than standard DTI [27]. Multimodal longitudinal imaging in presymptomatic cohorts may additionally help to establish if certain imaging indices exhibit early ceiling effect [127] which would limit its utility to track the post-symptomatic changes in clinical trials [136]. Robust presymptomatic studies can also deliver on important academic objectives. A myriad of environmental factors have been proposed in ALS which could be objectively evaluated in vivo if mutation carriers were tracked from a young age over multiple timepoints and environmental factors would carefully recorded.

Conclusions

From an academic perspective, presymptomatic studies offer invaluable learning opportunities to study propagation patterns, characterise early genotype-associated signatures, assess functional resilience, explore concepts like "motor reserve" or "cognitive reserve", and evaluate neurodevelopmental or environmental factors. However, with the advent of ASO therapies, the meticulous study of presymptomatic cohorts in ALS gained practical relevance and unprecedented urgency. Future studies have to be designed

Table 4 Lessons of existing presymptomatic studies in ALS, learning points for future study designs

Common shortcomings of existing studies	Desirable design features for future studies	Potential deliverables
Significant cohort size limitations	Several genotypes contrasted	Phenotypic indicators
Predominantly single-centre studies especially in relation to *SOD1*	Large samples, multicentre initiatives with standardised assessments, biomarker SOPs, inter-rater reliability testing, harmonised pulse-sequences for imaging	Disease onset estimation
Mixed presymptomatic cohorts of several mutations e.g. mixed *SOD1*/*C9orf72*	Multi-timepoint longitudinal study designs	Informing timing of ASO therapy
Admixed symptomatic cohorts (sporadic patients and mutation carriers)	Relatively uniform age at study entry	Evaluation of motor reserve
Symptomatic cohorts often included without genetic testing	Inclusion of mutation carriers in their late teens early twenties to assess for developmental factors	Evaluation of cognitive reserve
Scarcity of projects on mutations other than *SOD1*/*C9orf72*	Follow-up beyond symptom manifestation	Evaluation of adaptive mechanisms
Lack of 'disease controls' in the symptomatic group	Evaluation of disease burden at the time of fulfilling diagnostic criteria	Biomarker supported assessment of environmental factors in presymptomatic mutation carriers
Single modality studies (either imaging or wet biomarkers)	Combined wet biomarker imaging protocols	Exploration of epigenetic factors
Scarcity of spinal MRI studies	Inclusion of spinal imaging protocols	Refinement of current diagnostic criteria to integrate emerging biomarkers e.g. "Radiologically supported ALS"
Electrophysiology studies dominated by TMS studies, paucity of presymptomatic MUNE/MUNIX studies	Combined LMN-UMN electrophysiology protocols	Integration of emerging biomarkers into pharmacological trials
Limited clinical assessment	Study of gene-negative suspected ALS patients to characterise early changes in sporadic patients	Development of a framework for presymptomatic clinical trials before widespread degenerative changes ensue
Neuropsychological testing is often more detailed or better documented than neurological testing		
Cross-sectional or two-timepoint study designs		
Subjects not followed until symptom manifestation		
Subjects not followed until fulfilling diagnostic criteria		
Study entry in the mid-thirties or forties		
ALS-associated presymptomatic changes inferred from specific genotypes overlooking the fact the majority of ALS patients are sporadic		

to address specific clinical objectives such as informing the timing of pharmacological interventions, monitoring response to therapy, validating phenotypic indicators, and develop novel, biomarker-supported diagnostic criteria to facilitate earlier entry in clinical trials.

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Compliance with ethical standards

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