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Presymptomatic cognition and endophenotypic
traits of neurodegenerative disorders of the motor
system

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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Colm Peelo

This thesis is dedicated to the caregivers;
to mine, and to all those supporting people with
neurological conditions.

Summary

Neurodegenerative diseases of the motor system, such as Huntington's disease (HD) and Amyotrophic Lateral Sclerosis (ALS), are traditionally defined by their respective core motor symptoms. Research increasingly identifies a broader and more complex phenotype than previously recognised, which includes heterogeneous cognitive and behavioural presentations. The genetic nature of these disorders offers a unique window to study the manifestation of disease over time. Clinical neuropsychology plays a vital role in characterising extramotor symptoms and identifying early markers of disease, with implications for diagnosis, staging, prognosis, and clinical trial readiness.

This thesis employs comprehensive multi-domain neuropsychological assessment across several studies in ALS and HD to evaluate the utility of a transdiagnostic approach to phenotyping in early neurodegenerative disease stages, and endophenotyping for disease liability in at-risk populations. Cross-sectional and longitudinal designs enable the investigation of stable traits, disease progression, and the temporal evolution of cognitive and behavioural phenotypes.

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is the gold-standard cognitive screening tool in ALS, administered to each patient at their first visit to the National MND Clinic. The first study aimed to quantify and assess the effects of subjective distress on cognitive function in pwALS (n=60). Results showed that although levels of distress on the Distress Thermometer were high at a patient's first visit, they did not impact ECAS scores. Distress was associated with carer-reported symptoms of apathy however.

ALS has a complex genetic origin. 10% all cases and 40% of familial ALS (fALS), are caused by *C9orf72*. Previous work uncovered cognitive trait differences in unaffected first- and second-degree relatives of patients with familial ALS (fALS) which may constitute cognitive endophenotypes associated with unknown genetic mechanisms. In this thesis, two studies aimed to develop upon these novel findings. The first recruited a new sample of fALS relatives (n=67) and healthy control participants (n=37) to replicate the Phase 1 results. By repeating the neuropsychological assessment methods, results supported replication, particularly for confrontation naming. The second study reassessed a sample of fALS relatives (n=52) from Phase 1 to examine trait temporal stability, and also the effects of *C9orf72*-carriership over time. Over the 4-6 year interval in the longitudinal study, traits generally remained lower than control participants, but importantly, did not deteriorate. Neither did *C9orf72*-carriers display any differences from those without the gene. This again substantiates the initial findings and furthers consideration of these traits as putative cognitive endophenotypes.

HD-Cognition is a longitudinal study of cognitive and behavioural change in premanifest and early-HD. The study focuses on social cognitive and executive function, endeavouring to characterise the earliest extramotor symptom presentation. While the symptomatic manifest HD (manHD) participants displayed a globally reduced cognitive profile, premanifest HD (preHD) participants exhibited subtle signs in psychomotor processing speed and working memory which differentiated them from control participants. Social cognitive scores were associated with estimates of disease progression however. The longitudinal aspect confirmed sensitivity of social cognitive tasks to progression, as preHD performed worse over time on these, and on speed-based executive tasks.

Apathy is common in ALS and HD, and has shown association to cognitive function and prognosis. The profile of apathy and its cognitive correlates were examined in pwALS (n=171) and PwHD (n=44; n=33 controls) using the Dimensional Apathy Scale (DAS). Cohorts displayed differing apathy profiles with Initiation apathy common to both, and Executive apathy also high in PwHD. Domain-specific apathy corresponded to pre-hypothesised cognitive domains, indicative of a mechanistic overlap aligning with neuroanatomical underpinning. These profiles were stable over time in the HD cohort.

There is no validated cognitive screening tool for HD, in which the first presentation is often non-motor symptoms. The ECAS is widely used in ALS. As cognitive domains are highly relevant to both, there is much potential for utility in HD. Two versions of the ECAS (A & B) were administered at consecutive timepoints in the HD-Cognition study alongside the extended neuropsychological battery. The ECAS showed excellent convergent validity to the gold-standard on the majority of domains, on both versions. Sensitivity and specificity were equal to the original validation in ALS for the primary domains, and it also demonstrated sensitivity to longitudinal change on Reliable Change Indices. The ECAS, although has limitations, performs well in PwHD. Further validation is required, but it shows high suitability for use in HD.

In conclusion, this thesis presented a number of studies which contribute to the neuropsychological characterisation genetic liability in ALS, and of asymptomatic gene carriers and early manifest neurodegenerative disease over time. The broader aim supports the advancement of a domain-based, dimensional model of cognitive and behavioural change in heterogeneous neurodegenerative disease, with ALS and HD as exemplars. The importance of valid and reliable assessment of extramotor function throughout disease stages is emphasised with implications for trial sample enrichment, clinical care, and non-motor diagnostic criteria in HD.

Works from this thesis

Oral Presentations:

1. **PSI DoN Neuropsychology Symposium and Early Career Event** – Dublin, Ireland, September 2024. Poster and online presentation. *“Cognitive endophenotypes in familial ALS: a longitudinal study”*.
2. **PSI DoN Neuropsychology Symposium and Early Career Event** – Dublin, Ireland, September 2024. Poster and online presentation. *“Cognitive performance and distress in the ALS clinic”*.
3. **ENCALS Meeting** – Stockholm, Sweden, June 2024. Platform presentation. *“Cognitive endophenotypes in familial ALS: a longitudinal study”*.
4. **ENCALS Meeting** – Barcelona, Spain, June 2023. Rapid fire & Poster presentation. *“Investigating cognitive endophenotypes and presymptomatic cognition in ALS: Longitudinal cognitive performance of unaffected familial ALS relatives and C9orf72-gene-expansion carriers”*.
5. **ENCALS Meeting** – Edinburgh, Scotland, June 2022. Platform presentation: *“Examining the nature of phonemic verbal fluency in the Familial ALS cognitive endophenotype”*.
6. **PSI Annual Conference** – Portlaoise, November 2022. “Cognition in Neurodegenerative Diseases” Symposium Presentation: *“Apathy and verbal fluency in ALS: the chicken and egg of this sensitive cognitive biomarker”*.
7. **PSI Annual Conference** – Online, November, 2021. “Cognition in Neurodegenerative Diseases” Symposium presentation: *“The Edinburgh Cognitive ALS Screen as Sensitive and Specific Measures of Cognitive and Behavioural Dysfunction in Huntington’s Disease”*.

Poster Presentations

1. **INS Global Neuropsychology Congress** – Porto, Portugal, July 2024. Poster presentation. *“Cognitive endophenotypes in familial ALS: a longitudinal study”*.
2. **ENCALS Meeting** – Stockholm, Sweden, June 2024. Poster presentation. *“Cognitive performance and distress in the ALS clinic”*.
3. **MNDA International Symposium on ALS/MND** – Basel, Switzerland, December 2023. Poster presentation. *“Cognitive endophenotypes in familial ALS: a longitudinal study”*.
4. **ENCALS Meeting** – Barcelona, Spain, June 2023. Poster presentation. *“An investigation of Neurological Soft Signs in unaffected relatives of people with familial ALS: exploring an expanding endophenotype of neurological dysfunction among ALS kindreds”*.
5. **EHDN Plenary Meeting** – Bologna, Italy, September 2022. Poster presentation. *“Apathy in three dimensions: incidence and neuropsychological correlates of apathy in HD”*.

PPI & stakeholder engagement

1. **HD PPI Event**, December 2024.
2. **HDAI AGM – Invited speaker**, October 2022.
3. **HDAI “Meet the HD Researchers” Webinar**, May 2021.

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List of Abbreviations

ACS-SP — Advanced Clinical Solutions: Social Perception Battery	DAS — Dimensional Apathy Scale
ALS — Amyotrophic Lateral Sclerosis	DLPFC — Dorsolateral prefrontal cortex
ALS-FTD — ALS with Frontotemporal Dementia	DPR — Dipeptide Repeat proteins
ALS-FTSD — ALS Frontotemporal Spectrum Disorder	DSB — Digit Span: Backwards
ALSbi — ALS with Behavioural Impairment	DSF — Digit Span: Forwards
ALScbi — ALS with Cognitive and Behavioural Impairment	DTI — Diffusion Tensor Imaging
ALSci — ALS with Cognitive Impairment	ECAS — Edinburgh Cognitive and Behavioural ALS Screen
ALSFRS-R — ALS Functional Rating Scale Revised	EEG — Electroencephalography
ALSn — ALS Normal Cognition/behaviour	EMG — Electromyography
ANT — Action Naming Test	FAB — Frontal Assessment Battery
AQ — Autism Spectrum Quotient	fALS — Familial ALS
ASRS — Adult ADHD Self-Report Scale	fALS relative — unaffected at-risk biological relative of fALS patient
AUDIT — Alcohol Use Disorders Identification Test	FrSBe — The Frontal-Systems Behavioural Scale
BBI — The Beaumont Behavioural Inventory	FSIQ — Full Scale Intellectual Quotient
BIS — Barrett Impulsiveness Scale	FTD — Frontotemporal Dementia
BNT — Boston Naming Test	FTLD — Frontotemporal Lobar Degeneration
bvFTD — Behavioural-Variant Frontotemporal Dementia	FVC — Forced Vital Capacity
C9+/C9- — C9orf72-positive/negative	GAD — Generalised Anxiety Disorder
C9orf72 — Chromosome-9, Open Reading Frame-72 — the most common genetic cause of ALS and FTD	GAM — Generalised Additive Model
CAG — Cytosine-Adenine-Guanine (the expanded trinucleotide repeat on the <i>HTT</i> gene)	GHQ — General Health Questionnaire
CAP — CAG-Age-Product	GWAS — Genome-wide association
CAPE — Community Assessment of Psychic Experiences	HADS — Hospital Anxiety and Depression Scale
CBD — Corticobasal Degeneration	HTT / <i>HTT</i> — the huntingtin protein / the huntingtin gene
CLT — Central Limit Theorem	ICC — Intra-Class Correlation
CNS — Central Nervous System	IGT — Iowa Gambling Task
CWIT — Colour-Word Interference Test	IQ — Intellectual Quotient
D-KEFS — Delis-Kaplan Executive Function System	IQR — Interquartile Range
	LMM — Linear Mixed-Effects Model
	LMN — Lower Motor Neurons
	mHTT / <i>mHTT</i> — mutant huntingtin protein / gene
	ML — Maximum Likelihood Estimation
	MNAR — Missing-not at random

MND — Motor Neuron Disease	WAIS-IV — Wechsler Adult Intelligence Scale (Version 4)
MoCA — Montreal Cognitive Assessment	WASI-II — Wechsler Abbreviated Scale of Intelligence (Version 2)
mPFC — medial prefrontal cortex	WGS — Whole Genome Sequencing
MRI — Magnetic Resonance Imaging	WMS-III — Wechsler Memory Scale (third edition)
NCD — Neurocognitive disorder	
NPV — Negative Predictive Value	
OCI-R — Obsessive-Compulsive Inventory Revised	
PBP — Progressive Bulbar Palsy	
PD — Parkinson's disease	
PET — Positron Emission Tomography	
PFC — prefrontal cortex	
PHQ-9 — Patients Health Questionnaire-9 item	
PLS — Primary Lateral Sclerosis	
PMA — Progressive Muscular Atrophy	
PPA — Primary Progressive Aphasia	
PPV — Positive Predictive Value	
PSP — Progressive Supranuclear Palsy	
PSQ — Psychosis Screening Questionnaire	
pwALS — person with ALS	
PwHD — persons with HD	
RAVLT — Rey Auditory Verbal Learning Test	
RCFT — Rey Complex Figure Test	
RCI — Reliable Change Index	
REML — Restricted Maximum Likelihood Estimation	
RFI — Relative Fluency Index	
RMET — Reading the Mind in the Eyes Test	
sALS — Sporadic ALS	
SART — Sustained Attention to Response Task	
TDP-43 — TAR DNA-Binding Protein 43	
TIPI — Ten Item Personality Inventory	
TOPF — Test of Premorbid Functioning	
TOST — Two One-Sided t-test	
UNM — Upper Motor Neurons	
VFI — Verbal Fluency Index	
VIF — Variance Inflator Factor	

1 Chapter 1:

Introduction to Amyotrophic Lateral Sclerosis & Huntington's disease

1.1 Introduction to ALS

Amyotrophic Lateral Sclerosis (ALS; OMIM 105400) is a rapid and relentlessly progressive neurodegenerative disorder of the motor system. It is a motor neurone disease (MND) characterised by degeneration of both the upper (UMN) and lower motor neurones (LMN).¹ It was originally classified as a pure neuromuscular disorder, but it is now well established that it is a multisystem disorder, also affecting the non-motor neuraxis.^{2,3} ALS is also on a clinical, pathological and genetic spectrum with Fronto-Temporal Dementia (FTD; OMIM 600274), which primarily presents with cognitive and behavioural change as a result of fronto-temporal lobar degeneration (FTLD).

Upper motor Neurones (UMNs) are those of the primary motor cortex and which project through the cortico-spinal tract to the spinal cord and brainstem (**Figure 1**). Lower Motor Neurones (LMNs) include the anterior horn cells in the spinal cord and cranial nerve nuclei in the brainstem, which innervate skeletal muscle directly. Progressive degeneration of these UMN and LMN cell groups in ALS cause characteristic upper- and lower-motor neurone signs.⁴ The ALS clinical profile is one of progressive muscle wasting, limb weakness, bulbar dysfunction and respiratory failure, with symptom-onset and evolving symptom profile dependant on the location and spread of pathology.

1.1.1 *Clinical features of ALS*

There are three widely-recognised onset types in ALS determined by first presenting symptom; spinal-onset, bulbar-onset and respiratory onset. Spinal onset ALS is the most common form (up to 60% of cases).⁵ It is classified by weakness or wasting of muscles in the limbs. Lower-limb spinal onset can present as 'foot-drop', stiffness or

heaviness in the legs or unsteadiness, which make patients more likely to trip or fall. Upper-limb onset presents with weakness of the hand or arm, difficulty with fine-motor tasks and poor grip-strength. Both progress to the point where patients can no longer walk or use their upper limbs to function. This onset-type is most common in men.^{3,6}

Bulbar onset ALS presents in about 33% of cases⁵, most typically women and older-onset cases.^{3,7} First symptoms involve difficulty with motor-speech (dysarthria) or swallowing (dysphagia) due to weakness of the muscles in the tongue, mouth or throat. Respiratory onset ALS is the third main onset-subtype, characterised by weakness of the muscles controlling breathing. Symptoms include dyspnoea (shortness of breath), orthopnoea (difficulty breathing when lying down) and unexplained weight loss. It is the least common (5%) and tends to have the shortest prognosis, along with bulbar onset.

A sub-type of spinal onset ALS are variants which present with symmetrical LMN signs confined to the limbs. These are known as segmental “flail-limb” variants. Flail arm syndrome involves symmetrical LMN weakness in both arms, causing shoulder wasting and flaccid upper limbs; it is more common in men.^{1,8} Flail leg syndrome affects both legs distally. These variants are rare, progress slowly, and are associated with median survival of 8+ years.¹

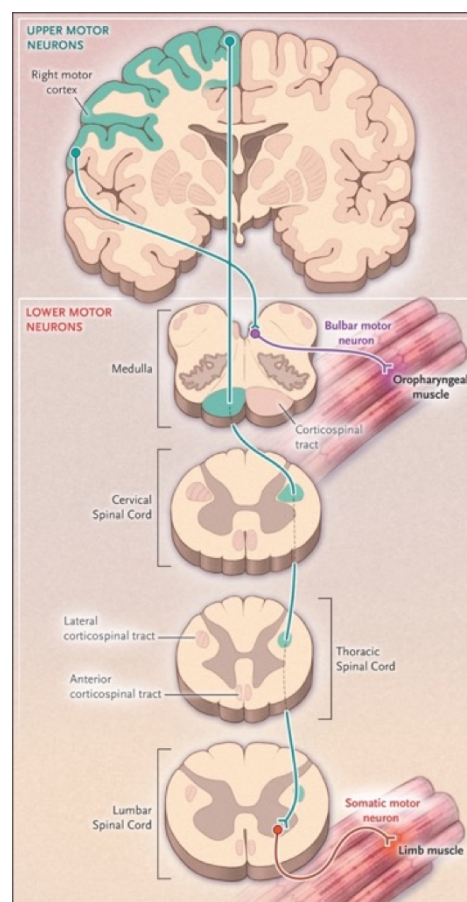


Figure 1: The motor system, including neurones of the primary motor cortex, corticospinal tract and lower motor neurones.

From Brown and Al-Chalabi, 2017.⁴

1.1.2 ALS diagnosis

Early symptoms of ALS are often nonspecific, shared by many neurological disorders and ALS-mimics. Without reliable and specific biomarkers, diagnostic delay is protracted, as ALS is a diagnosis of exclusion. The El Escorial criteria (EEC) were first established in 1994 and revised in 1999, to provide a structured, standardised approach to ALS diagnosis.⁹ The revised framework categorises patients along a continuum of diagnostic certainty ranging from “possible” to “definite” ALS based on the number of body regions affected, the presence of upper and/or lower motor neuron (U/LMN) signs, and the inclusion of supportive neurophysiological evidence (“lab supported probable”). The criteria have been criticised for excluding cognitive and behavioural subtypes, delaying diagnosis and limiting the representation of these phenotypes in clinical research and therapeutic trials.¹⁰ Other diagnostic criteria include Awaji and Gold Coast Criteria, but the EEC are the most commonly used.¹¹

1.1.2.1 Cognitive-onset ALS

Whilst cognitive-onset is sometimes considered a fourth diagnostic criteria of ALS, it is rarer, and not widely recognised alongside spinal, bulbar and respiratory onset categories. People with ALS (pwALS) presenting first with cognitive symptoms often have a longer diagnostic delay than classical motor onset ALS.¹² Further complicating the diagnostic process, early symptoms of frontotemporal dementia-ALS (FTD-ALS) can include psychiatric features, such as depression, hallucinations and delusions.¹³⁻¹⁵

Cognitive and behavioural dysfunction are now well recognised symptoms of ALS, with up to 50% of cases presenting with some degree of cognitive/behavioural change over the disease-course.² Approximately 35% have mild symptoms, with 15% meeting criteria for FTD at some point in the disease course. The Strong Criteria, revised in 2017¹⁶, provide a framework with which to characterise cognitive and behavioural symptoms in ALS- Frontotemporal spectrum disorders (ALS-FTSD). Not typically used as part of general diagnostic criteria (EEC), they supplement the motor diagnostic criteria by specifying four extramotor subtypes (**Table 1**).

1.1.3 Differential diagnoses, other MND subtypes & clinical overlap with ALS

ALS is diagnosed clinically, characterised by its progressive course and the involvement of both UMN and LMNs across multiple body regions. Few conditions mimic

this presentation, making the differential diagnosis relatively limited. The clinical features of ALS overlap with many other neurological disorders, including FTD and atypical parkinsonian syndromes, like Corticobasal syndrome (CBS) and Progressive Supranuclear Palsy (PSP).¹⁷ The key clinical features that help distinguish ALS from other MND disorders and relevant differential diagnoses are presented here.

Table 1: Strong Revised Diagnostic Criteria. From Strong et al. (2017).¹⁶

Subtype	Criteria
ALSci	Either executive dysfunction (including social cognition) <u>or</u> language dysfunction <u>or</u> a combination of the two. Executive Impairment defined as: Impaired verbal fluency (letter). OR Impairment on two other non-overlapping measures of executive functions (which may include social cognition) Language impairment is defined as: Impairment on two non-overlapping tests and in which language impairment is not solely explained by verbal fluency deficits.
ALSbi	Apathy with or without other behaviour change OR two non-overlapping supportive diagnostic features from the Rascovsky criteria^{a18}
ALScbi	meet ALSci and ALSbi criteria
ALS-FTD	1. Progressive deterioration of behaviour and/or cognition by observation or history AND 2. At least 3 behavioural/cognitive symptoms outlined by Rascovsky¹⁸ OR 3. At least 2 of those behavioural/cognitive symptoms, with loss of insight and/or psychotic symptoms OR 4. Language impairment meeting criteria for semantic dementia/semantic variant PPA or non-fluent variant PPA (by Gorno-Tempini et al.)¹⁹

ALSbi = ALS with behavioural impairment; ci = cognitive impairment; cbi = cognitive and behavioural impairment; FTD = frontotemporal dementia.

^a *disinhibition, apathy/inertia, loss of sympathy/empathy, perseverative/compulsive behaviours, hyperorality, dysexecutive neuropsychological profile.*

1.1.3.1 Other MND subtypes

ALS is one of many under the umbrella of motor neurone diseases. Other non-ALS MND include primary lateral sclerosis (PLS), primary muscular atrophy (PMA), and progressive bulbar palsy (PBP).^{1,20}

PLS is defined by UMN degeneration without LMN involvement or muscle wasting. Symptoms typically begin in the lower limbs, with onset around age 50. Up to half of PLS patients eventually show LMN signs and transition to ALS. Therefore, recommendations are that a diagnosis of PLS is contingent upon 4 years without LMN involvement to ensure diagnostic certainty.²¹ Until then, it may be referred to as UMN-predominant ALS.^{1,8} PLS has a relatively favourable prognosis, $\geq 20+$ years for those who do not transition to ALS, and comprises less than 1% of MND cases.^{5,20}

PMA is the opposite of PLS, in that it involves isolated LMN degeneration with UMN-sparing. It primarily affects men, with onset usually after age 50.^{1,8} It accounts for 5–10% of MND cases overall. Although initially confined to LMN signs, around 30% of PMA cases later develop UMN involvement and are therefore diagnosed as ALS. Such cases, may be referred to as LMN-predominant ALS. Prognosis is more variable in PMA, but generally more favourable than ‘classical-ALS’. Depending on location of disease onset, survival can be five years and up to 20 from symptom onset.⁵

PBP is defined by speech and swallowing difficulties due to LMN degeneration restricted to the bulbar region. It accounts for roughly 20% of MND presentations and is more common in older women. Most patients develop UMN- and wider LMN-involvement, leading to an ALS diagnosis. PBP is often considered an early stage of a bulbar-onset ALS variant rather than a distinct disorder.⁵

1.1.3.2 ALS-FTD

ALS shares genetic, molecular, pathological, and anatomical features with FTD.²² As a result, they are considered part of the same continuum of disease, and more recently are phenotypes conceptualised within the ALS–frontotemporal spectrum disorder (ALS-FTSD) umbrella.^{16,23} The ALS-FTD spectrum includes phenotypes with pure-motor involvement, i.e.: classical-ALS, to an intermediary of ALS with cognitive and/or behavioural change, to a pure cognitive/behavioural FTD without motor features (**Table 1**). Clinico-pathological associations in FTD-MND are high, supporting its nosological validity as a disorder spectrum.

The proportion of patients with ALS (pwALS) who meet clinical criteria for FTD is similar to the number with FTD who subsequently develop features of ALS, about 15%.^{22,24}

Up to 50% of FTD patients may meet criteria for at least possible ALS, by El Escorial Criteria.²⁵ Most commonly in the ALS/FTD overlap, frontotemporal dysfunction and an FTD diagnosis precedes ALS (FTD-ALS); in others, ALS appears first (ALS-FTD), or both may emerge simultaneously.^{24,26} Primary extramotor symptoms in ALS-FTD overlap syndrome are behavioural changes (e.g.: disinhibition, apathy, loss of empathy, inappropriate social behaviour, neglect, verbal and motor stereotypies, altered food preferences and behaviours)¹⁸, but presentations with predominantly language impairment with symptoms akin to mild lvFTD, have also been reported.²⁷ Evidencing the strength of the overlap, extramotor diagnostic criteria for ALS, revised in 2017 by Strong et al.¹⁶, use the Rascovsky criteria for behavioural variant FTD (bvFTD)¹⁸ as deterministic of ALS behavioural impairment.

1.1.3.2.1 Corticobasal syndrome & Progressive Supranuclear Palsy

Corticobasal syndrome (CBS), named by its neuropathological hallmark, Corticobasal degeneration (CBD), shares similarities with ALS. CBS typically presents with cortical and extrapyramidal signs, including asymmetric limb apraxia, rigidity, dystonia, postural instability, cortical sensory loss, and the characteristic “alien limb phenomenon”.²⁸ Progressive Supranuclear Palsy (PSP) also presents with some features of ALS and CBS, characterised by parkinsonism, supranuclear gaze palsy, and pronounced retropulsion. Presentations in both CBS and PSP may also include pseudobulbar features such as dysarthria, dysphagia, and emotional lability, common in ALS.^{29,30} With increasing awareness of extra-motor symptoms in motor disorders across clinical neurology, behavioural and cognitive impairments are now recognised in both CBS and PSP.³⁰⁻³³ These include many of the key diagnostic features of language-variant FTD (lvFTD) and bvFTD, as well as neuropsychiatric features.³⁴ PSP and CBS are tauopathies within the pathological frontotemporal lobar degeneration (FTLD) spectrum which also includes FTD and Alzheimer’s disease (AD).^{28,29,33}

1.1.3.3 ALS progression & prognosis

ALS has a rapid disease course, with the duration from symptom onset to diagnosis (diagnostic delay) usually between 10 to 16 months, and from diagnosis to death, typically one to four years.^{3,35} The disease course is highly variable however. 25% of patients live beyond 5 years, and 5–10% survive over a decade. In Ireland, mean survival is approximately 2.4 years from onset and 1.3 years from diagnosis.³⁶ Over time, patients lose fine motor control, become unable to speak or swallow, and eventually require communication aids and enteral feeding. Respiratory failure is ultimately the cause of

death, with symptoms like dyspnoea, fatigue, and sleep disturbances arising from hypoventilation and gas exchange abnormalities occurring at any stage.⁵

Prognostic algorithms have been developed which aim to determine disease duration.³⁷ Negative prognostic indicators include respiratory onset, bulbar onset, weight loss, executive function deficits and FTD diagnosis.^{3,6,38} The order of presentation of cognitive dysfunction may convey survival.³⁹ Conversely, younger onset, longer diagnostic delay, and higher lipid levels may be linked to slower progression and extended survival.^{5,35,40}

Staging systems were devised to define disease progression with a view to support clinical judgement, monitoring severity and guiding treatment. The two most common are the the Kings and Milano-Torino (MiToS) staging systems. They define disease stage based on the number of areas (of four) affected and the ALS Functional Rating Scale-Revised (ALSFRRS-R) subscores respectively.⁴¹ Higher stages correspond to progression and more disability. Stage 4 in the King's staging system is met when the pwALS requires either gastrostomy or respiratory support, typically non-invasive ventilation (NIV).

The ALSFRS-R is a rating scale, typically administered by a clinician, which quantifies the function of a pwALS on 12 items across four domains (bulbar, spinal upper-limb, spinal lower-limb, respiratory).⁴² The maximum scores is 48, with higher scores indicative of better functional ability, although domain scores offer the best descriptive ability.⁴³ It is often used as a proxy for disease progression in clinical trials and research studies.

1.1.4 Epidemiology of ALS

ALS is a rare disease. The incidence globally is estimated at approximately 0.6 to 3.8 per 100,000 person-years.⁴⁴ Annual incidence in Europe is 2-3 per 100,000 adults with a prevalence of approximately 5.2 per 100,000, and is lowest in Asian populations.^{1,45} In Ireland, the annual incidence and prevalence are approximately 2.5-3.1 per 100,000 person-years and 6.8 per 100,000 persons respectively.^{36,46,47} There have been trends of increasing incidence and prevalence in the literature.^{44,48} A recent study of 25 years of the Irish MND register discovered incidence and prevalence had increased during this time as well as age of onset (AoO), although survival is unchanged.⁴⁸ Lifetime risk for ALS is estimated to be 1 in 350 for men and 1 in 400 for women.¹ Risk increases with age, typically presenting between 50 and 75 years of age.⁵

1.1.5 Pathophysiology of ALS

ALS is a syndrome with multiple aetiologies, and therefore pathophysiology varies. It does not have a single pathological hallmark, and much of the pathogenesis remains unknown.⁴⁹ Universal to all forms of ALS is axonal dysfunction and subsequent death of upper and lower motor neurons.⁵⁰ As with most neurodegenerative diseases, ALS can be caused by aggregations of mis-folded proteins in neuronal cells, produced by aberrant genes which also may be unable to perform their usual function.⁵⁰ Pathogenesis can be governed by the different genetic contributors to ALS, which cause distinct neuropathological signatures. For example, *SOD1*-related ALS typically lacks TAR DNA-binding protein 43 (TDP-43) pathology, whereas the vast majority of ALS cases display cytoplasmic inclusions of TDP-43, which is encoded *TARDBP*.^{20,51} This mislocalisation of TDP-43 is present in up to 97% of ALS cases.^{20,51} Similar to the pathological hallmark for Alzheimer's disease, amyloid-beta deposition, TDP-43 cytoplasmic inclusions are present in up to 40% of people over the age of 65 who have no neurological symptoms, and so it is neither a sensitive nor specific biomarker.⁵¹ However, regional deposition of TDP-43 has been shown to map onto performance on cognitive screening tools.⁵²

Hardiman et al.³ published a comprehensive overview of the background to ALS.

Figure 2 depicts several key pathways and the action of mutated genes which disrupt cellular functions, leading to axonopathy in motor neuron cells.³ Disrupted protein homeostasis (proteostasis) and abnormal RNA processing are among the most prominent pathological processes, both of which are linked to multiple ALS-associated gene mutations. While all MND subtypes, outlined above, share core pathological features, their anatomical distribution varies, thus producing variable phenotypes.^{20,53} **Figure 3** shows the theoretical process whereby cumulative damage from genetic load, environment and time combine to produce a self-perpetuating cascade of pathology and expression of the ALS clinical phenotype, should a threshold be reached.¹

1.1.6 Genetics of ALS

ALS is a clinical syndrome with multiple aetiologies, including multiple genotypes, which produce a broadly similar phenotype (**Figure 4a**). About 10% of ALS cases have a known family history and heritability is estimated at approximately 52%.⁴⁷ Heritability is a measure how much the risk of developing a disorder or condition can be attributed to a person's genetic makeup. In an Irish ALS population, studies show penetrance is lower, about 37%, when patients test negative for *C9orf72*.⁴⁷ Others' estimate heritability

between 40 and 60%, concordant with the Irish sample.^{54,55} Despite the discovery of 40 gene variants associated with the condition (see Alsultan et al., 2016 for review^{56,57}), much remains unknown about the genetics of ALS. **Figure 6a** shows a discovery timeline (until 2012) of the genes implicated in ALS-FTSD and where they fall in terms of contribution to phenotype along this spectrum. Heritability estimates are not fully explained by known genetic mechanisms and so ALS likely has a complex genetic origin.⁵⁸

Most forms of the condition are thought to be oligogenic, caused by the combination of a few genes of large effect.^{1,59,60} Although large numbers are required to elucidate the genomic mechanisms in complex genetic disorders, such as ALS, they may be underpinned by simpler genetics that give rise to endophenotypes. A recent series published in *The Lancet Neurology* reviewed the current understanding of four of the main genetic forms of ALS—*C9orf72*⁶¹, *SOD1*⁶², *FUS*⁶³, and *TARDBP*.⁵¹ *C9orf72* is the most prevalent of these in Ireland, and is particularly important for this thesis.

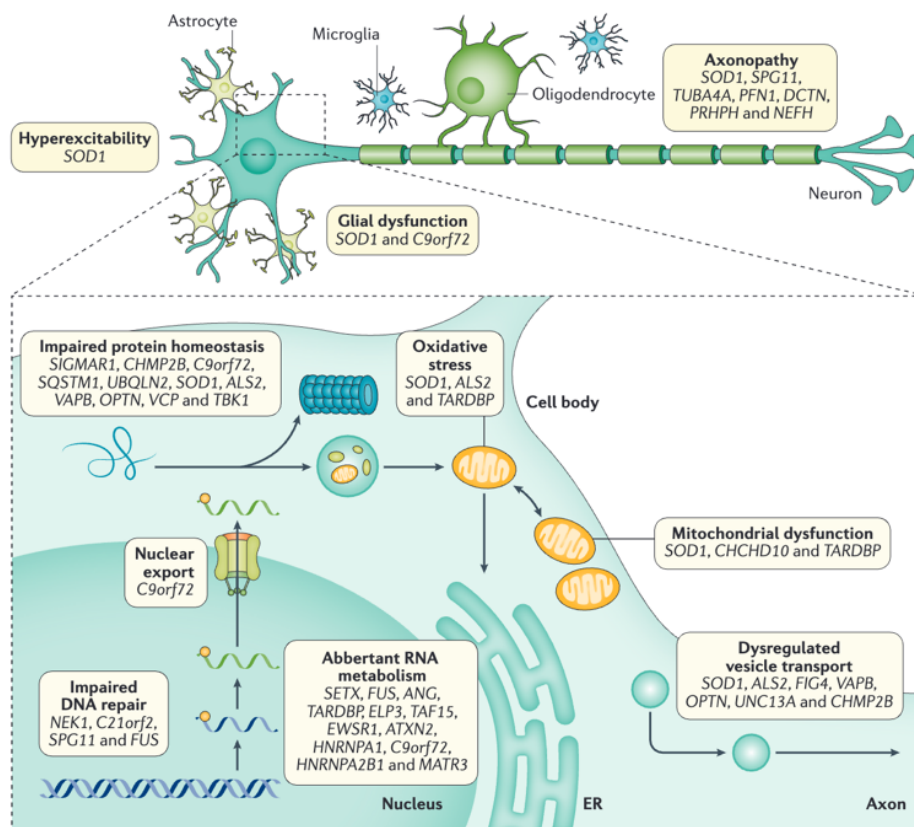


Figure 2: The pathophysiology of ALS, from Hardiman et al. (2017).³

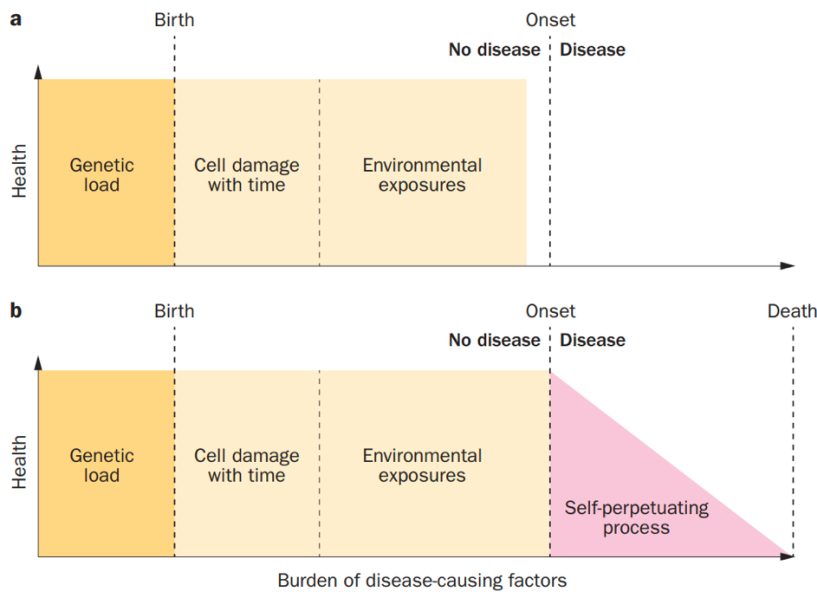


Figure 3: ALS risk factors for disease onset.

Theoretical sequence leading to disease onset in ALS, a) showing a person who does not develop ALS, indicative of incomplete penetrance of the ‘genetic load’, and b) a threshold reached, beginning a self-perpetuating disease process (from Al-Chalabi & Hardiman, 2013).¹

1.1.6.1 C9orf72

A hexanucleotide (GGGGCC) repeat-expansion (HRE) on Chromosome-9, Open Reading Frame-72 (*C9orf72*) is responsible for a large proportion of the genetic causes of ALS and FTD. The range of repeat expansions to be considered ‘*C9orf72*-positive’ can vary. Initial assessment found expansions of 30 or more repeats to be pathogenic, but it is generally accepted now that 24 or more repeats can produce the disease phenotype, with 20-23 considered in the intermediate range.⁶⁴⁻⁶⁷

C9orf72 accounts for almost 40% of familial ALS (fALS) and 25% of familial-FTD and approximately 5-11% of sporadic cases (i.e.: cases with no known family history), comprising approximately 10% of incident ALS cases in Ireland.⁶⁶⁻⁶⁸ *C9orf72* is autosomal dominantly inherited but is not fully penetrant.^{69,70} Throughout this thesis, carriers of the *C9orf72*-HRE in the pathogenic range are referred to as *C9orf72*-positive (C9+), and those at-risk for carriership, but who do not carry the expanded gene are referred to as *C9orf72*-negative (C9-).

1.1.6.1.1 C9orf72 penetrance & pleiotropy

Genetic penetrance is the probability that a specific trait or phenotype is expressed given a particular genotype or set of genetic variants.^{71,72} Penetrance estimates for many ALS/FTD-causing genes (*C9orf72*, *SOD1*, *FUS*, *TARDBP*) are much lower than initially

reported from studies of retrospective disease cohorts, which were thought to be near 100% by a carrier's mid-80s.^{73,74} Revised estimations from recent population-based studies range from 24.1% at age 80 for first-degree relative carriers to 44% in the general population who carry the repeat expansion.^{72,74-77} Penetrance is also incomplete and highly variable between *C9orf72*-ALS kindreds, with estimates ranging from 16-61% between families.⁷⁷ Low penetrance suggests there are other genetic and environmental precipitants which lead to disease manifestation.

The *C9orf72*-gene variant is also pleiotropic meaning that this one gene can cause multiple different phenotypes (**Figure 4b**).^{69,70} **Figure 6b** further illustrates this, showing the overlap between FTD and ALS, and a number of alternative pathological and clinical outcomes of the *C9orf72*-HRE, including a number of phenocopy disorders where it has been implicated.⁷⁸ Additionally, *C9orf72* has shown genetic overlaps with schizophrenia.⁷⁹

The pleiotropic effects and incomplete penetrance of the *C9orf72* gene-variant suggests there are many unknown genetic factors involved in disease pathogenesis and ALS is likely a complex, oligogenic disorder.^{1,59,60} To illustrate incomplete penetrance for the dominantly inherited genotype, only 20.3% of *C9orf72*-positive pwALS in Ryan et al.'s study had a parental history of ALS.⁴⁷ Conversely, in 39.1% of parent child-dyads diagnosed with ALS, the child (proband) did not carry any known mutations. Further evidence for complex oligogenic mechanisms within *C9orf72*-families is the presence of genetic discordance. Gene-negative pwALS within gene-positive kindreds suggest alternative genetic mechanisms are increasing disease liability alongside the *C9orf72* repeat expansion in these kindreds.⁸⁰ A large longitudinal imaging study tracking changes to cortical thickness mapped the patterns of participants who developed ALS during the study.⁸¹ The similarity models they developed predicted that less than 30% of the unaffected C9+ relatives would show similar cortical patterns at age 80, thus implying a reduced level of genetic penetrance.

1.1.6.1.2 *C9orf72* pathophysiology

Although uncertainty remains, the pathomechanism of *C9orf72* is thought to be a complex combination of gain- and loss-of-function.⁶¹ Expanded repeats produce bidirectional repeat RNA toxicity and *C9orf72* haploinsufficiency (loss-of-function of wild-type protein). Pathological hallmarks are dipeptide repeat protein (DPR) deposition and TDP-43 mislocalisations in neuronal cells.

There is no consensus on whether the length of the hexanucleotide repeat expansion bears any significance on age at onset or disease severity in ALS.^{82,83} Dols-Icardo

et al.⁸⁴ found no relationship between the length of the repeat expansion in the *C9orf72* gene and clinical phenotype, age of onset or prognosis. They reported variability in the expansion size within individuals in different tissues (genetic mosaicism) and between individuals within families.

C9orf72 may have a neurodevelopmental effect, creating a lifelong vulnerability to disease until significant accumulation of other risk factors produce a pathological cascade leading to neurodegeneration and disease onset (**Figure 3**).^{1,85-87} The imaging study mentioned above also showed reduced gyrification and smaller volumes of cortical and subcortical brain structures in unaffected *C9+* carriers, compared to unaffected *C9-* relatives at 40 years of age, unrelated to disease-associated pathology.⁸¹ The multi-step hypothesis of ALS highlights the combination of oligogenic mechanisms (multiple genes of medium effect), time, environment, gene-gene and gene-environment interaction effects which combine to produce the ALS syndrome (see **Figure 5**).⁸⁶ The accumulation of disease-causing factors in the context of this vulnerability may be what triggers disease onset in ALS and FTD.¹

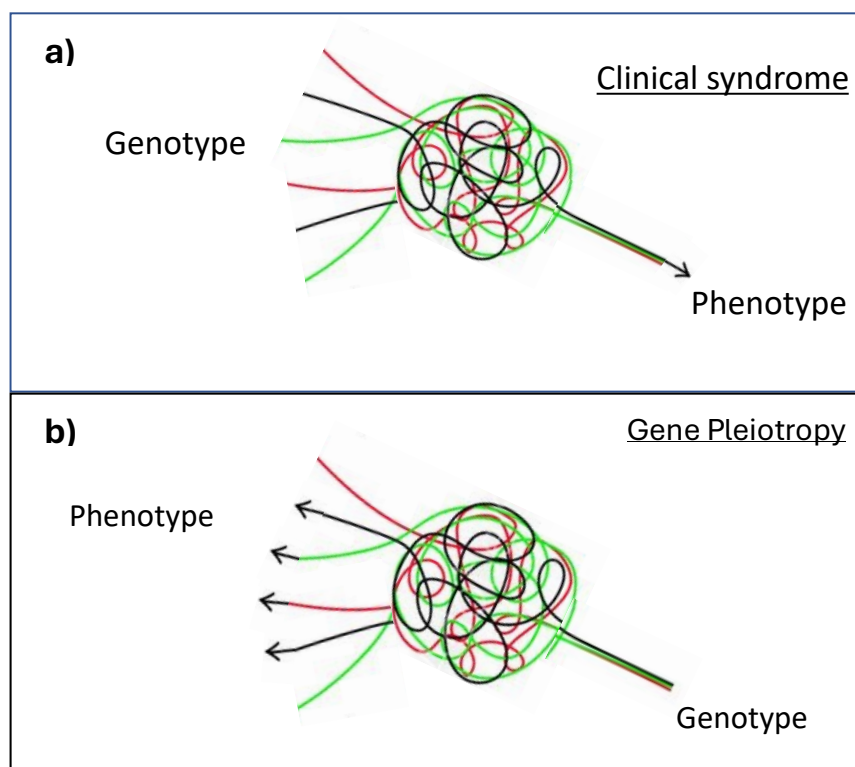


Figure 4: Competing genotype/phenotype interaction phenomena in ALS. Schematic of, a) Clinical syndrome, & b) gene pleiotropy.

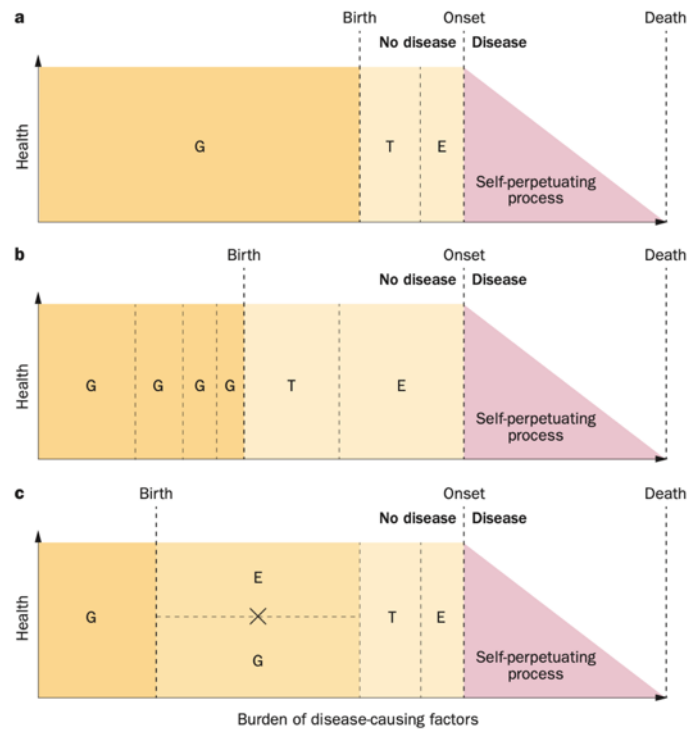


Figure 5: Gene x Time x Environment model of ALS.

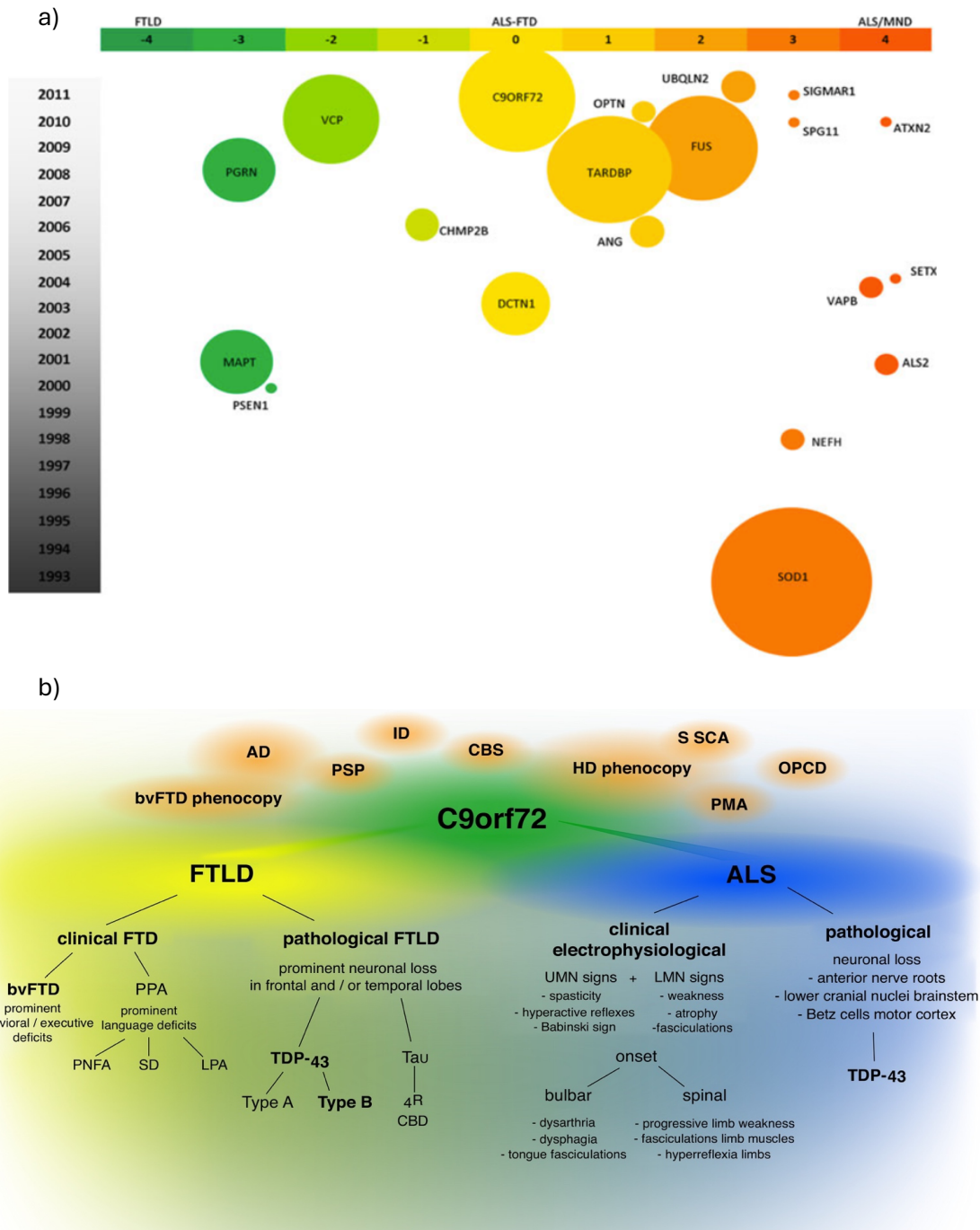
Interaction of genes and environment which contribute to ALS onset with different magnitude of effects from, a) single gene of large effect, b) multiple genetic influences of smaller effect (oligogenic), and c) a gene-environment interaction effect (from Al-Chalabi & Hardiman, 2013).¹ G = gene / genetic load, T = Time, E = Environment, E X G = Environment-gene interaction.

1.1.6.2 SOD1

Multiple mutations of the copper-zinc superoxide dismutase (*SOD1*) gene are causative of ALS.⁶² They produce a primarily motor-phenotype which begins in younger patients. *SOD1* mutations vary across populations, between 1–6%⁸⁸, and are rare in Ireland. *SOD1*-ALS demonstrates a prion-like propagation of pathology, causing a toxic gain-of-function.

1.1.6.3 FUS

Fused in sarcoma (*FUS*) is a mutation to the gene producing the *FUS* RNA-binding protein. It causes a young-onset, rapidly progressive form of ALS and a small proportion of FTD cases.⁶³ Mutation to the *FUS* gene are rare, present in 0.3% of all pwALS. But it is dominantly inherited and so is present in approximately 2.8% of fALS cases. *FUS*-ALS is molecularly distinct from other forms of ALS. A unique pathological hallmark is basophilic cytoplasmic inclusions which are negative for TDP-43.⁶³ These aggregates produce a toxic gain-of-function, resulting in cell death.



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Figure 6: ALS Genetics.

a) a timeline of gene discovery and the magnitude of the impact of common genes associated with the ALS-FTD spectrum (from Al-Chalabi et al.⁵⁰); **b)** the pleiotropic effects of C9orf72 and associations to neurological disorders, highlighting the overlap between ALS and FTD. From Van Mossevelde et al.⁷⁸

1.1.6.4 TARDP & others

Although TDP-43 cytoplasmic inclusions are the most common pathological hallmark of ALS, only a minority of ALS cases have a mutation to the *TARDDBP* gene.⁵¹ It is

affected by low specificity, but may be a more universal target for intervention in ALS and FTD. Other genetic factors implicated in ALS pathogenesis are Angiogenin (AGN-1), Vasopressin (VCP), Microtubule Associated Protein-Tau (MAPT) and GRN. The latter two (MAPT and GRN) are also causative of FTD.^{60,89,90}

1.1.7 Other risk factors for ALS

The aetiology of ALS is complex with a large proportion of risk due to age and heritability. Definitive evidence for environmental risk factors has yet to be found. However, there are a number which may contribute to the development of ALS. Wang et al.⁹¹ carried out a systematic review of the evidence for environmental risk factors in ALS with the strongest links to heavy metals exposure, pesticides and other organic chemicals, and certain traumas, including electric shock and head trauma.

Emerging evidence linking FTD and ALS to traumatic brain injury (TBI) and chronic traumatic encephalopathy (CTE) in the case of repetitive subclinical or mild-TBI insults. Increased rates of ALS is seen in players of contact sport and at the extreme ends of physical exercise. Cognitive impairment and psychiatric symptoms have been observed at increased rates in retired professional American football players⁹², and head trauma has been proposed as a risk factor for ALS, FTD, AD and PD.^{93,94} Strenuous physical activity has also been linked to increased risk for developing ALS.⁹⁵⁻⁹⁷

1.1.8 The clinical C9orf72 phenotype

The *C9orf72* repeat expansion is associated with a severe ALS phenotype. It is associated with higher prevalence of cognitive and behavioural symptomatology, earlier onset and a shorter prognosis.^{1,68} Rates of FTD in pwALS with the HRE are more than four times those without. *C9orf72*-positive patients also have an increased prevalence of psychiatric traits and symptoms.^{68,98} A recent study across the FTD disease-span has shown neuropsychiatric and behavioural symptom severity and frequency were highest in symptomatic *C9orf72*-FTD patients compared to *GRN* and *MAPT* groups in general, with symptoms beginning long before clinical disease onset. Psychotic symptoms presented in *C9orf72*-carriers at much higher rates than any other genotype.⁹⁹ Deficits on verbal fluency performance for *C9orf72*-positive patients have been shown across conditions (ALS, FTD and ALS-FTD patients).¹⁰⁰ *C9orf72*-HRE alone may not produce a disease phenotype. A neurodevelopmental vulnerability, plus the DNA effects of somatic expansion, time and

environmental effects, in combination with other oligogenic mechanisms are likely requisite for the disease phenotype.

1.1.9 *Treatments for ALS*

Riluzole was the only medication with proven efficacy in slowing ALS progression until recently. 100mg produces modest survival benefit and slower muscle strength decline, with a 2–3 month increase in survival and a 9% improvement in one-year survival, with adequate safety and tolerability.¹⁰¹ Despite its limited effect and high cost, Riluzole remains widely recommended due to the lack of alternatives. While the precise mechanism is unclear, it is thought to modulate glutamate excitotoxicity, supporting neuronal survival. Edaravone is a free radical scavenger originally used for stroke treatment, repurposed for use in ALS. It may work to reduce oxidative stress, however, the clinical value remains contested due to limited follow-up, lack of biomarkers, and inconsistent results in broader ALS populations.¹⁰² It is not widely prescribed in the EU.

Tofersen, or Qualsody (BIIB067), is an Antisense Oligonucleotide (ASO) gene therapy which has shown great efficacy for treating a specific mutation in *SOD1*-ALS. It works by reducing the SOD1 protein and as a result, neurofilament biomarkers in the cerebrospinal fluid (CSF), resulting in a slowing of symptom progression in carriers.^{103,104} Another ASO showing early promise is Jacifusen. It works by silencing the faulty *FUS* gene, binding to and disabling *FUS* mRNA.¹⁰⁵ Phase 3 clinical trials evaluating the efficacy of Jacifusen are currently being carried out.

ASOs have their limitations, and are genotype specific. Trials of ASOs in *C9orf72*-ALS on the other hand have not been successful.^{106,107} There is a disconnect between pharmacodynamic success and clinical outcomes in trials for ASOs in *C9orf72*-ALS. Given the likely influence of other oligogenic effects it points to the need for combinatorial or upstream interventions that target the yet unknown steps in the multi-step hypothesis of ALS.^{61,86}

1.1.10 *A note on familiality in ALS*

The diagnosis of ALS can also refer to whether or not a pwALS has a positive family history for ALS and/or FTD. The risk of developing ALS in Ireland increases from 0.26% to 0.7% for a person with a family history of ALS where the proband has no known causative genes.⁴⁷ It increases further to 1.4% for relatives where proband genetic status is unknown, and further still if the patient proband is *C9orf72* positive. Despite this, the distinction

between familial and sporadic ALS is useful, but somewhat arbitrary, and therefore flawed. Familiarity has been discussed up to this point as a diagnostic category of ALS, whereby those with a positive family history for ALS are considered fALS and those with no apparent family history are ‘apparently sporadic’ ALS (sALS). Byrne et al.¹⁰⁸ improved this loose distinction, standardising criteria for the definition of familiarity in ALS in 2011 (see **Table 2**). Determining whether a pwALS is familial is confounded by a number of factors that make this distinction artificial: small family size, incomplete ascertainment, low awareness or accuracy for extended family history, low penetrance of large effect genes, improvements in life expectancy.^{60,108} Empirical support from this comes from the Irish ALS register where newly presenting, apparently sALS cases are re-categorised as fALS at a rate of about 3% per year due to this “information creep”.⁴⁷ Categorising ALS cases as ‘sporadic’ may be temporally bound and limited by the information available at the time. It may only be descriptive of the probands’ recall and declarative family history. Truly sporadic pwALS who carry the *C9orf72* repeat expansion are likely rare. The majority of these cases have a family history of other neurodegenerative (dementias) or neuropsychiatric diseases highlighting the pleiotropic effect of *C9orf72* and possibly wider unknown genetic effects.¹⁰⁹

Byrne’s criteria acknowledge this and go beyond categorisation by family history alone. The inclusion of genetic status in the definition of fALS removes the possibility of a gene-positive sALS cases. These cases are most likely subject to the biases mentioned above, and are better considered ‘Possible’ fALS.¹⁰⁸ It makes categorisation more objective, especially considering the fallibility of patient-reported family histories. This has been mostly adopted by the ALS research community, and is increasingly being considered in clinical settings also. **Figure 7** shows a timeline of gene discovery by relative contribution to familial and sporadic cases of ALS. It shows *C9orf72* as the largest contributor to both familial and sporadic ALS cases in populations of European ancestry, with the second largest share in fALS and the largest in sALS currently unknown.⁸⁹ Uptake in clinical genetic testing in symptomatic pwALS has increased significantly due to demand for clinical trial participation. Not yet being performed in Ireland, demand for genetic testing in asymptomatic at-risk individuals has also increased globally in recent years.¹¹⁰⁻¹¹²

Table 2: The Byrne Criteria for familial ALS (fALS).

Proposed criteria for familial ALS combined from Byrne et al. (2011) and Ryan et al. (2018). pwALS come under the following categories of familiarity if they have:

Definite fALS	
History	at least two 1 st or 2 nd degree relatives with ALS.
Genetics	at least one relative with ALS and a gene-positive co-segregation. ^a
Probable fALS	
History	one 1 st or 2 nd degree relatives with ALS.
Possible fALS	
History	≥ one distant relative with ALS (≥ 3 rd degree).
FTD	a relative with confirmed Frontotemporal Dementia.
Neuropsychiatric	≥ three relatives with schizophrenia. ^b
Unspecified dementia	≥ two relatives with dementia. ^b
Genetics	no family history of ALS (apparently sporadic), but positive for a fALS gene. ^a

^a fALS gene, such as, *C9orf72*, *FUS*, *SOD1*, *TARDBP*. ^b Proposed by Ryan et al. (2018)¹⁰⁹. Included here for descriptive purposes. Not considered as fALS in subsequent studies.

NOTE: 1st degree relatives: parent, child or sibling. 2nd degree relatives: grandparent, aunt/uncle.

According to the Byrne criteria¹⁰⁸, the rate of fALS in Ireland is estimated to be as high as 20%, considerably higher than previous estimates of about 10%. The overlap with psychiatric and other neurodegenerative conditions in the ALS family history led Ryan et al.¹⁰⁹ to propose additional criteria for ‘possible fALS’ to include pwALS with family history of 2+ relatives with dementia and 3+ relatives with schizophrenia. Including Ryan et al.’s¹⁰⁹ proposed neuropsychiatric and dementia criteria in the definition (**Table 2**), rates of fALS increase to 30% in Ireland.

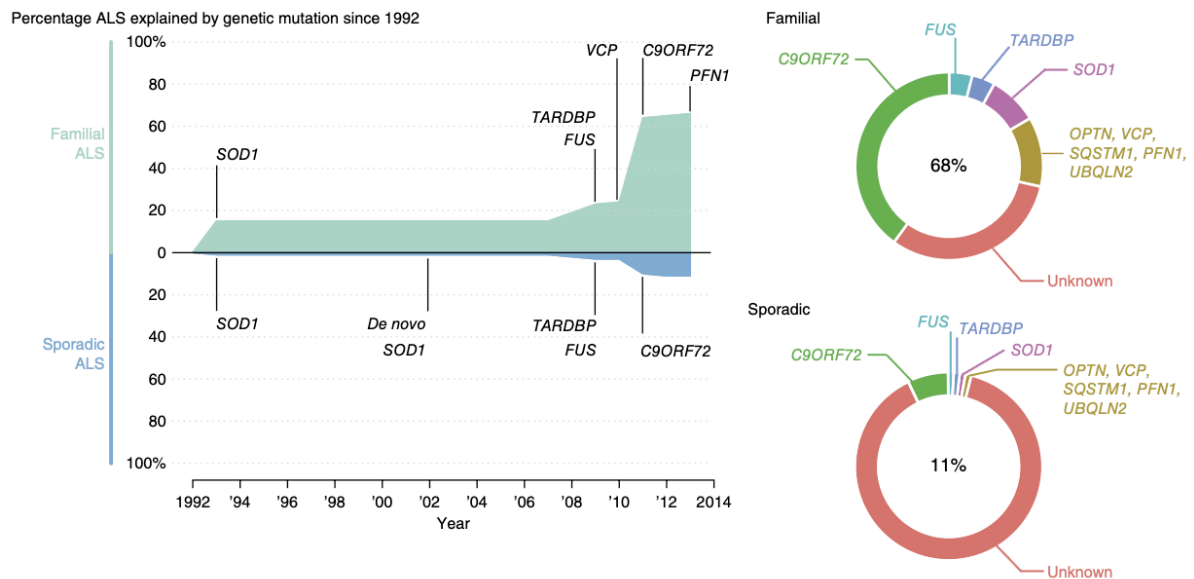


Figure 7: Genetic causes of familial- vs sporadic-ALS. From Renton et al. (2014).⁸⁹

1.2 The neuropsychology of ALS

Clinical, neuroimaging and pathological evidence of cognitive and behavioural involvement have been demonstrated for some years to be part of the ALS phenotype, confirming its status as a multi-system clinical syndrome. The Strong Criteria formalised the characterisation of ALS with cognitive and/or behavioural impairment (ALSc/bi).¹⁶ This neuropsychological profile reflects variable frontostriatal and temporal involvement and overlaps with the FTD phenotype.^{52,113} One of the first large-scale studies in ALS reported cognitive impairment in 51% of ALS patients, with 15% overall meeting criteria for FTD in their clinic-based sample¹¹⁴, rates of impairment mirrored by a large population-based study of incident ALS cases performed in Ireland.² Up to 50% of pwALS demonstrate no cognitive or behavioural impairment at any stage in the disease course.^{23,115}

Behavioural and cognitive symptoms present heterogeneously in terms of profile and order of presentation. They may precede, coincide with or follow motor symptom onset.⁴⁵ Executive dysfunction is the most common profile, in particular, impairments in phonemic verbal fluency reflecting dorsolateral-prefrontal cortex (DLPFC) dysfunction.^{2,116-118} More diverse prefrontal dysfunctions are also evident, with impairment to cognitive flexibility, reasoning, working memory, and executive inhibition.^{2,16}

A meta-analysis showed strong evidence for impairments in language, verbal fluency, executive function, and social cognition in ALS.¹¹⁹ This study reported moderate effect sizes for verbal fluency, language, and social cognition, and smaller effects for executive tasks, but also for verbal recall.¹¹⁹ An update which included more studies

showed concordant results, with the largest effect sizes for verbal fluency, social cognitive function, and executive function more generally.¹¹⁵ The most reliable period which demonstrated these effects was for disease durations of 18 to 24 months, with relatively low heterogeneity across studies in all domains.¹¹⁵ pwALS also show reduced performance on confrontation naming language tasks.^{23,115,119}

Behavioural symptoms observed in FTD are also common, including disinhibition, loss of empathy, stereotypy, dietary change, and most prominently, apathy, which is reported in up to 50% of cases, likely reflecting anterior cingulate involvement.^{23,24,120} Cognitive and behavioural symptoms have shown concordance to the regional spread of TDP-43 pathology in post-mortem brain tissue.⁵²

1.2.1 Executive dysfunction in ALS

Executive function is the umbrella term given to a collection of discrete processes that modulate and co-ordinate complex goal-directed cognitions, such as planning, set-switching, response inhibition, problem solving and the initiation and execution of complex actions^{121,122}, coordinated by the supervisory attentional system (SAS) and central executive components of working memory.^{123,124} Executive dysfunction is a core cognitive symptom in ALS, reflecting disruption of fronto-striatal and prefrontal networks. Deficits on tasks such as the Stroop interference and Brixton spatial anticipation tests highlight difficulties with response inhibition and cognitive flexibility. These impairments are often linked to degeneration in the dorsolateral prefrontal cortex (DLPFC) and are associated with clinical symptoms such as apathy and disinhibition.^{23,125} Errors of executive inhibition are common, but less severe than bvFTD, shown by difficulty generating unrelated word completions on the Hayling sentence completion task. Attentional impairment and poorer performance on dual-task paradigms requiring divided attention, which have shown correlation with structural alterations in frontal regions in pwALS.¹²⁶ Real-life decision-making tasks have also shown decreased connectivity in medial cingulate and other prefrontal areas.

1.2.2 Social cognition (ALS)

Social cognitive deficits, particularly in theory of mind (ToM) and emotional recognition, are increasingly recognised in ALS.^{119,127,128} A meta-analysis by Finsel et al.¹¹⁵ demonstrated medium-to-large effect sizes for deficits in social cognition, highlighting these impairments as prevalent early in the disease course. ALS patients have difficulty inferring emotional states and intentions from social cues^{129,130}, particularly eye gaze¹³¹, and making

social as compared to non-social judgements.¹³² These deficits correlate with reduced connectivity in the frontal-temporal networks, implicating the default mode network and regions like the anterior insular and cingulate cortices.^{23,133} The anterior insula is a principal afferent hub of the salience network, which underlies exogenous, or bottom-up, attentional capacity. This area has also been implicated in facial affect recognition and moral decision-making in FTD.¹³⁴

1.2.3 Verbal fluency performance in ALS

Phonemic verbal fluency task performance appears to show selective impairment in ALS.^{118,135,136} Deficits are most consistently reported as a characteristic hallmark of ALS.¹⁶ Phonemic verbal fluency is differentially affected, with the relative sparing of performance on semantic fluency tasks. These tasks tap overlapping but discrete neurobiological pathways. Phonemic verbal fluency performance requires a complex interplay of executive and linguistic processes relating to generation and switching, and retrieval from the mental lexicon¹³⁷⁻¹⁴⁰, and thus requires recruitment of many brain structures including left inferior frontal gyrus, the DLPFC, anterior cingulate cortex (ACC), inferior frontal gyrus, or Broca's area.^{141,142} More than the sum of its parts based on research aiming to assess the latent constructs that combine in phonemic verbal fluency¹¹⁸, higher order task requirements include executive (i.e.: initiation, updating, inhibition, monitoring), phonological and lexical processes within working memory. Neuroimaging studies of phonemic verbal fluency performance highlight chiefly the DLPFC and medial prefrontal cortices (mPFC), and the inferior frontal and anterior cingulate gyri as important for the task, along with motor and premotor regions.^{117,143} White matter integrity along the superior longitudinal fasciculus and corpus callosum are also implicated.^{23,116,144} Subtle alterations in any of these networks can affect task performance.

Semantic fluency recruits lexical-semantic memory, mostly underpinned by temporal lobe semantic networks, with less fronto-executive demands, corroborated by neuroimaging studies.¹⁴⁵⁻¹⁴⁷ Function in the SAS likely underlies this discrepancy in task performances.^{118,148}

Motor impairment can significantly confound cognitive test scores in ALS, especially timed tasks such as verbal fluency. The verbal fluency index (VFI) controls for this.^{118,149} Although some studies report levels of impairment as low as 9% in pwALS when VFI is used¹⁵⁰, imaging studies have shown focal fibre tract disruption between the corpus callosum and supplementary motor area as relevant to impairment.¹⁵¹ PET studies support

these regions as important in ALS, which found areas of hypometabolism in bilateral DLPFCs, insular cortices and anterior thalamic nuclei.¹⁴³

1.2.3.1 Behavioural change in ALS

Behavioural changes are commonly observed among pwALS.^{152,153} Apathy, particularly Initiation apathy, is the most prevalent symptom, reported in up to 45% of cases.¹⁵⁴ Other behavioural changes include disinhibition, loss of empathy, perseverative behaviours, and hyperorality, paralleling behavioural variant frontotemporal dementia (bvFTD) symptoms.^{23,152,155} Abrahams (2023)²³ notes these behavioural changes occur across the ALS-FTD spectrum and correlate with frontal-temporal cortical dysfunction. A systematic review found perseveration most characteristic of severe behavioural impairment in pwALS, with 8% of cases overall meeting criteria for bvFTD.¹⁵⁶ Other neuropsychiatric symptoms have been shown to present before motor symptoms in ALS without FTD, with no effect on survival.¹⁵⁷ Five distinct behavioural phenotypes in ALS arose from principal component analyses which were hypothesised to correlate with disruptions to specific neural networks, namely the frontostriatal, orbitofrontal, and ACC.¹⁵⁸ This evidence suggests clinically relevant behavioural subgroups exist with neuroanatomical correlates, challenging the notion of a singular, homogeneous behavioural presentation in ALS, and with psychiatric symptoms as early indicators of disease in some cases.

1.2.4 Screening for ALS-related cognitive impairment

The Edinburgh Cognitive and behavioural ALS Screen (ECAS) was created as a quick and repeatable screening tool for ALS-related cognitive impairment, which crucially, controls for motor-impairment.¹⁵⁹ It is the most widely used tool for cognitive screening in ALS and can be performed in verbal or written format. It evaluates five domains, three of which, language, fluency, and executive function, comprise an ALS-specific score. Memory and visuospatial abilities contribute to an ALS-non-specific score, as these domains are generally spared. Alternate ECAS versions (B and C) support repeated assessment and longitudinal tracking of cognition for pwALS and can be administered every 4 months in serial order.¹⁶⁰ The authors demonstrated that these forms mitigate practice effects¹⁶¹, however, other studies demonstrate an improvement between A and B in particular.¹⁶² Demographically stratified normative data (by age and education) and measures of clinically significant and reliable change controlling for practice effects have been published in Ireland for all three serial forms (A-B-C).^{162,163}

1.2.5 Decline with advancing disease stage

Cognitive impairment appears to be more frequent in advanced stages of ALS. A cross-sectional study across Irish, Scottish, and English cohorts found that 80% of pwALS at King's stage 4 were impaired¹⁶⁴, compared to 64% in an Italian cohort.¹⁶⁵ These findings are limited by the absence of longitudinal follow-up however.

1.3 ALS cognitive function over time

The clinical neuropsychological picture of ALS over time remains unclear. Many studies have demonstrated cognitive and behavioural impairment at baseline.^{2,115,119} The majority of longitudinal studies show no change to cognitive function over time however, or the absence of practice effects.¹⁶⁶⁻¹⁶⁹ In most longitudinal studies included in Finsel et al.'s¹¹⁵ meta-analysis, cognitive performance remained stable across timepoints (62%), but this is often limited by non-random drop out, or attrition bias.

Elamin et al.¹⁷⁰ was the first large-scale population based longitudinal study of cognitive function in ALS. They found impairment at baseline, which remained stable, although attrition was high, suggesting impairments emerge early in the disease course. Those with early deficits showed faster disease progression and shorter survival in general. Cognitive, and particularly executive impairment, was a strong negative prognostic indicator.¹⁷⁰ Woolley et al. (2018)¹²⁰ report no cognitive change on the ALS-Cognitive Behavioural Scale (ALS-CBS) in a sample of pwALS over 12 months (n=134), but in whom behavioural deterioration was significant.

Two more recent studies from Italy^{167,171} completed full neuropsychological assessment as well as the ECAS in pwALS. Bersano et al.¹⁶⁷ reported cognitive decline in 32% of patients over six months, including some who were cognitively intact at baseline. Iazzolino et al.¹⁷¹ demonstrated similar rates of decline from baseline to follow-up (30% one year later). This occurred mostly in those who were impaired at baseline, either behaviourally (ALSbi) or cognitively (ALSci), or both (ALScbi). 21% of cognitively normal pwALS at baseline exhibited some degree of extramotor impairment at follow up (ALSc/bi/FTD).¹⁷¹ Neither Bersano et al.¹⁶⁷ nor Iazzolino et al.¹⁷¹ appear to use indices controlling for motor impairment in the standard neuropsychological assessment however, which can impact cognitive assessment outcomes. Improving upon this, Beeldman et al.¹⁷² did find a decline in cognitive function over a 6 month interval in pwALS, unrelated to motor or psychiatric function.

McHutchison et al.¹⁷³ also demonstrated baseline impairment and longitudinal decline in a small subset of pwALS, at much lower rates than expected (8% for both). pwALS who declined fell into two categories, 1) abnormal at baseline and likely age-related decline mostly on ALS-non-specific domains, or 2) were abnormal across all ECAS domains, related to *C9orf72* carriership.

A large imaging study with 630 measurements from 293 pwALS did not find decline of ECAS scores, even with alternate forms (A-B), but grey matter volume reductions were evident longitudinally.¹⁶⁹ Atrophy in regions associated with cognitive/behavioural functions were directly related to cognitive scores on the ECAS and decreased verbal fluency was most highly associated with diffuse white matter impairment¹⁶⁹, consistent with previous findings of the decreased integrity of the superior and inferior longitudinal fasciculi in ALS.^{116,144}

Divergent findings in longitudinal and cross-sectional designs underscore uncertainty regarding the natural course of cognitive decline in ALS. Few have robustly evaluated whether or not cognitive function declines in ALS longitudinally. Cross-sectional studies may conflate stage-related differences with true longitudinal change, whereas, clinical factors in the rapid disease course of ALS (i.e.: disease progression, disability, death) severely impact attrition in longitudinal studies and may underrepresent patients who decline most rapidly. Motor disability can also confound, or make impossible standard testing.^{174,175} These disease-related challenges impede conclusions about the natural disease course of cognition in ALS.

1.3.1 Neuroimaging extramotor impairment in ALS

Neuroimaging studies have shown a diverse and heterogeneous picture in ALS. Widespread cortical pathology of frontal, parietal and temporal regions correlate with varying cognitive presentations of ALS at different stages.^{113,176,177} Basal-ganglia and fronto-striatal atrophy may underlie the particular executive, social cognitive and apathetic profile in ALS_{Sci}.¹⁷⁸ Atrophy in thalamic structures are associated with *C9orf72*-ALS¹⁷⁹, and uniquely right thalamic atrophy with psychotic symptoms in *C9orf72*-FTD.⁹⁹

Patterns of atrophy described in the ACC, cingulate gyri, and orbitofrontal and superior temporal areas in patients with ALS_{Sci}^{180,181} Fronto-temporal atrophy has also associated with executive and behavioural impairments in ALS and ALS-FTD patients.¹⁸² Distinct sub-classifications of electrophysiological biomarkers have been demonstrated, relating to onset, survival and cognitive function in pwALS.¹⁸³ So too have alterations in

network connectivity and dysfunction been shown in asymptomatic *C9orf72*-gene carriers.¹⁸⁴ Patterns of cortical and subcortical grey matter atrophy and widespread white matter deterioration show domain specific patterns related to impairment on cognitive domains of the ECAS.¹⁶⁹ These patterns are shared between C9-positive and C9-negative pwALS, although are more pronounced in C9+.

Schönecker et al.¹⁸⁵ identified clusters of subclinical motor phenotypes in asymptomatic and symptomatic *C9orf72*-FTD, with distinct correlates of reduced cortical volume. Most common were ALS-like signs (77%), with parkinsonian and PSP-like presentations also. Motor signs in *C9orf72*-carriers appeared up to 25 years before expected onset. Lee et al.¹⁸⁶ were the first to study the effects of the *C9orf72* repeat expansion in asymptomatic carriers. This small sample (n=15) showed patterns of reduced grey matter and connectivity deficits similar to, but milder than C9+ pwALS from as early as their 30s. Despite the presence of these neuroimaging signals, cognitive function was no different from a sample of healthy controls. Others have reported memory deficits in pwALS which correlate with parahippocampal and hippocampal volume reduction.^{119,187} Overall, ALS demonstrates diffuse atrophy patterns, with regional associations to genotype (*C9orf72*) and cognitive impairment.

1.4 Family aggregation studies in ALS

Whilst *C9orf72* is associated with cognitive and behavioural symptoms in patients, ALS is associated with increased incidences of psychiatric and neurological presentations in the family history. ALS is recognised as an oligogenic disorder⁵⁹, meaning that even those without a known ALS-gene may carry other genetic variants that contribute to disease susceptibility. This complexity is highlighted by family aggregation studies, which find an increased risk of neuropsychiatric conditions, particularly psychotic disorders, and other neurodegenerative diseases among relatives of individuals with ALS¹⁸⁸⁻¹⁹² In all cases, relative-risk was elevated regardless of proband *C9orf72*-status, albeit to a lesser extent in C9-negative kindreds, when compared to unrelated healthy controls.^{188,189,191} McHutchison et al. (2020)¹⁹¹ described an association between increased psychiatric family history and cognitive/behavioural change in pwALS. In a family history study of ALS and FTD patients (n=89) including 1,400 individuals, Devenney et al.¹⁹⁰ found a ten-fold increase in relative risk for psychosis and schizophrenia in relatives of patients carrying the *C9orf72* mutation, compared to those without (**Figure 8**). Large Genome-Wide Association Studies (GWAS) report a shared polygenic risk between ALS and psychiatric disorders such as

schizophrenia, suggestive of shared aetiologies.⁷⁹ Therefore, while the pleiotropic effects of *C9orf72* are undoubtedly relevant, they do not account for the variation in the ALS family history nor the heterogeneity of the ALS/FTD phenotype

Polygenic risk from currently unknown genes appears to contribute to clinical variability.^{47,109,193} There are likely unknown genetic mechanisms that form a part of the oligogenic inheritance pattern and pathogenic process in ALS⁵⁹, both alongside and in the absence of *C9orf72*. Therefore, while cohort studies commonly employing control groups of familial-non-carriers may increase sensitivity to gene-specific effects, it potentially limits our ability to explore broader genetic factors that could be central to understanding disease mechanisms in ALS. For this reason, the study of kindreds and unaffected relatives of pwALS is valuable for uncovering other oligogenic or polygenic effects.

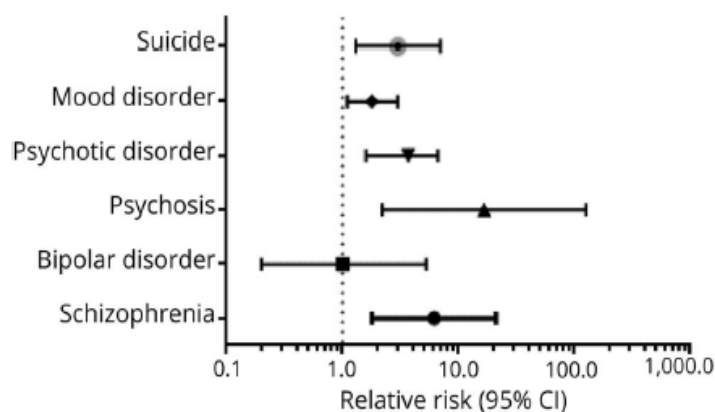


Figure 8: Relative risk of psychiatric disorders in *C9orf72* kindreds.

From Devenney et al. (2018).¹⁹⁰

1.5 The endophenotype concept

Previous work in our group has explored endophenotyping as a method to support gene-identification studies.¹⁹⁴ Endophenotypes are quantitative state-independent characteristics that connect genetic liability to phenotypic clinical disorder.¹⁹⁵ They are subclinical intermediaries which are heritable, associated with the illness and present at higher rates in patient kindreds than in the general population.¹⁹⁶ An endophenotype can provide insight into the manifestation of disorders of a complex genetic aetiology by linking discrete characteristics of disease associated with simpler genetic mechanisms which manifest subtle subclinical presentations.¹⁹⁶ There are many measurable biological functions and traits can be considered as endophenotypes, from motor and

neuropsychological function to transcriptomic markers.¹⁹⁷ In cases where there is evidence of familiarity but no detected disease-causing gene-variant, sub-clinical differences in family member characteristics, including psychiatric traits, motor function or cognition may prove useful as endophenotypes to elucidate genetic mechanism.

Endophenotypes link pathogenic processes associated with a distal genotype to the observable disease phenotype. As state-independent traits, they offer insight into the disorders with complex genetic aetiologies by connecting discrete, often subclinical characteristics to simpler or single-gene mechanisms (**Figure 9**).¹⁹⁶ As such, endophenotypes afford greater power to gene-discovery. According to Gottesman and Gould, a trait must meet five criteria to be considered an endophenotype: (1) association with the disease in the general population, (2) heritability, (3) co-segregation with the disorder within families, (4) independence from clinical state, and (5) higher prevalence in unaffected relatives than in the general population.¹⁹⁶ In the psychiatric literature, this has often included cognitive traits.^{195,198,199} The presence of an endophenotype is a marker, or genetic correlate, of increased disease liability in unaffected, at-risk relatives.²⁰⁰⁻²⁰²

In cases where there is evidence of heritability, but no detected disease-causing gene-variant, sub-clinical differences in family member characteristics, including psychiatric traits, motor function or cognition may prove useful as endophenotypes.^{199,200,203} The risk of developing ALS increases almost 5-fold for a person with a family history of ALS.⁴⁷ Therefore, relatives of patients with a family history of ALS are likely to have a higher genetic burden contributing to this risk, compared to the general population and sporadic relatives. Studying fALS relatives as a distinct subgroup in exploring the effects of endophenotypes will improve our understanding of the genomics and heritability of ALS.

1.5.1 Endophenotypes in neurological research: relevance to ALS

ALS pathogenesis is multifactorial, with multiple genetic origins and much remaining unexplained heritability.^{58,60} The multi-step hypothesis of ALS is described by Al-Chalabi et al.⁸⁶ and Chio et al.^{87,204} found ALS to require six steps to manifestation. Environmental factors and time influence and compound potential genetic factors in producing a disease phenotype. The number of steps is lowered by genes of large effect, such as *SOD1* and *C9orf72*, to three or four steps respectively.⁸⁶

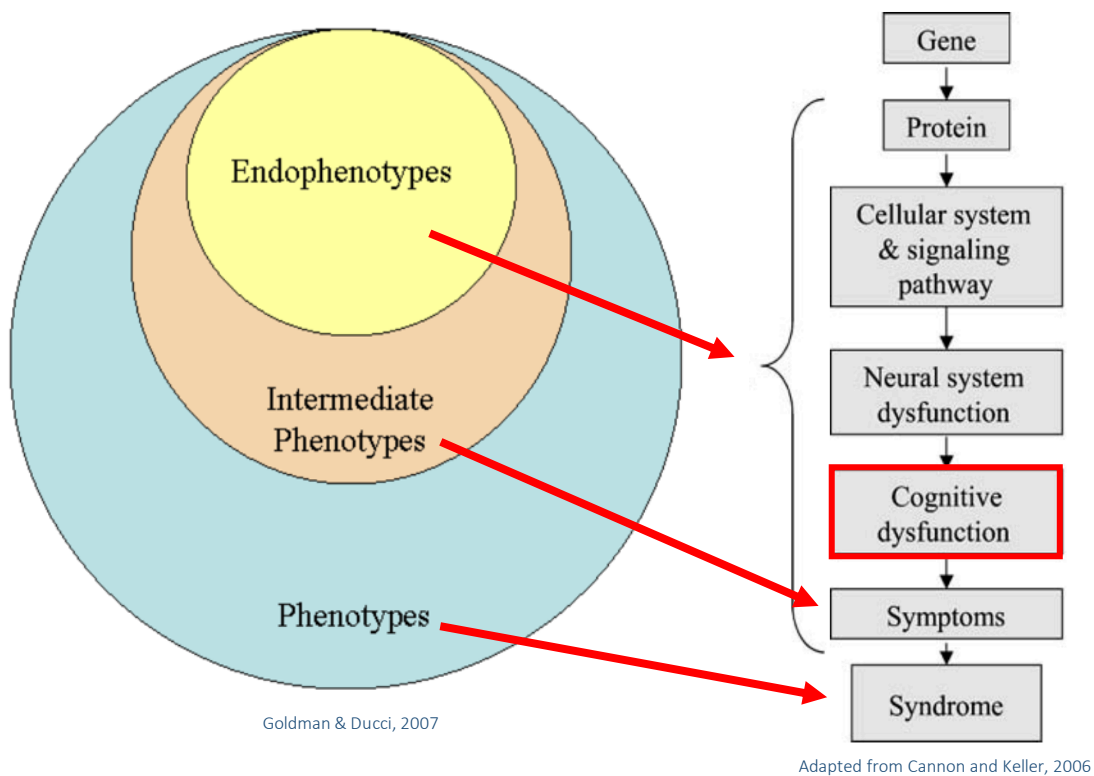


Figure 9: Categories of endophenotypes.

Schematic showing the levels at which endophenotypes can be found, including cognition. Adapted from Goldman & Ducci (2007) and Cannon and Keller (2006).¹⁹⁵

1.5.1.1 Cognitive endophenotypes in ALS

Cognitive endophenotypes are well described in the psychiatric literature.^{196,205,206} They have proven useful in disambiguating the complex genetic aetiology and heterogenous clinical presentations of schizophrenia in particular^{203,207}, as well as major depressive disorder²⁰⁸ OCD²⁰¹, and ASD.²⁰⁹ The diverse range of cognitive and behavioural changes observed in pwALS suggests that more than one neuropsychological endophenotype may contribute to the condition's clinical profile. Combined with the evidence of increased rates of neurological and psychiatric illness in unaffected relatives of pwALS, cognitive and personality trait differences in relatives of fALS patients provide strong evidence for the presence of neuropsychological endophenotypes for ALS.¹⁹⁴

A large family-based study in ALS recently demonstrated altered cognitive and personality traits in unaffected fALS patient-relatives compared to controls and relatives of sALS patients. Crucially, this was present irrespective of *C9orf72* status.^{194,210,211} The main finding of this work shows reduced performance on phonemic verbal fluency task in fALS relatives, but also for working memory, conscientiousness, and openness to experience personality traits.^{194,210} Given the selective impairment of phonemic verbal

fluency that is characteristic of ALSci^{16,23}, this finding proposes phonemic verbal fluency as a putative cognitive endophenotype for ALS, meeting Gottesman and Gould's criteria.¹⁹⁶ While individually non-specific and non-diagnostic, at the group level endophenotypic traits indicate a reduced disease-liability threshold in at-risk individuals. Further research is needed to clarify whether these candidate endophenotypes reflect stable, trait-like characteristics that could help identify new ALS-associated genes, or transient, state-like features possibly associated with disease progression.

1.5.1.2 Other potential endophenotypes in ALS

Neurological soft signs (NSS) are mild, non-disabling motor or sensory abnormalities detectable on detailed neurological examination.^{212,213} They are present in approximately 5% of the healthy population and tend to increase with age. Their presence may reflect subtle, diffuse neurological dysfunction, holding promise as a transdiagnostic marker.²¹⁴ In schizophrenia, a higher burden of NSS in early illness has been associated with poorer prognosis and more severe disease subtypes.²¹² Critically, NSS are also observed at elevated rates in unaffected first-degree relatives and phenotypically discordant monozygotic twins, suggesting their potential as a heritable endophenotype.²¹³ Subtle motor and cognitive-motor differences between *C9orf72* carriers and non-carriers have been described in the FTD literature, with carriers showing poorer praxis²¹⁵ scores and clinical motor scores, albeit in the subclinical range.¹⁸⁵ Translating these findings to ALS, as with cognitive endophenotypes, NSS may serve as a low-cost, clinically accessible marker of latent disease-liability, early-stage neurodegeneration, or possibly neurodevelopmental traits.

1.6 Introduction to Huntington's disease

Huntington's disease (HD; OMIM 143100) was first properly characterised by George Huntington in 1872, whose father and grandfather had been observing patients with this "hereditary chorea" since 1797.²¹⁶ His initial descriptions detailed a hereditary disorder of movement and psychiatry, initially given the title of 'Huntington's Chorea'; the namesake of its discoverer and the primary symptom. Though recognising HD was primarily a movement disorder, Huntington also noted the psychiatric, and cognitive/behavioural symptoms which together formed a unique clinical picture. Huntington described the three corner stones of HD as: "*Its hereditary nature. 2. A tendency to insanity and suicide. 3. Its manifesting itself as a grave disease only in adult life*". His characterisation of this unique clinical profile which was onset in middle age, and which followed autosomal dominant inheritance patterns through affected families galvanised HD as a discrete disorder. He described, prior to the widespread knowledge of Mendelian inheritance, the inheritance patterns through families. Patient's suffering from Huntington's chorea were affected by an unrelenting movement disorder, increased incidence of "insanity" and suicide, and in his earliest descriptions, an impulsivity and disinhibition.²¹⁷ Huntington also noted increased incidence of suicide and psychiatric symptoms in the patients and kindreds of persons with HD (PwHD), but who themselves did not necessarily have the disorder indicating a prodromal phase (*Huntington, 1872, pp. 320–321*).^{216,217} The gradual decline of behaviour and cognition contribute to a progressive decline in functional status throughout the disease course, impacting patient independence, occupation and education, and damaging interpersonal relationships.

1.6.1 Clinical features of HD

HD is a fully penetrant, autosomal dominant neurodegenerative choreiform movement disorder caused by an trinucleotide poly-glutamine (CAG) repeat expansions of the gene coding for the huntingtin protein (HTT). HD causes marked and progressive degeneration of structures within the central nervous system (CNS) giving rise to the triad of symptoms underlying the condition: hyperkinetic motor dysfunction, psychiatric/behavioural disturbance and cognitive impairment.²¹⁸ Whilst nearly all PwHD present with impairments across the three domains over the disease course, the severity and order of presentation varies between patients.²¹⁹

Motor symptoms in HD are generally characterised by a progression from subtle voluntary motor impairments to more overt involuntary movements.²²⁰ Involuntary

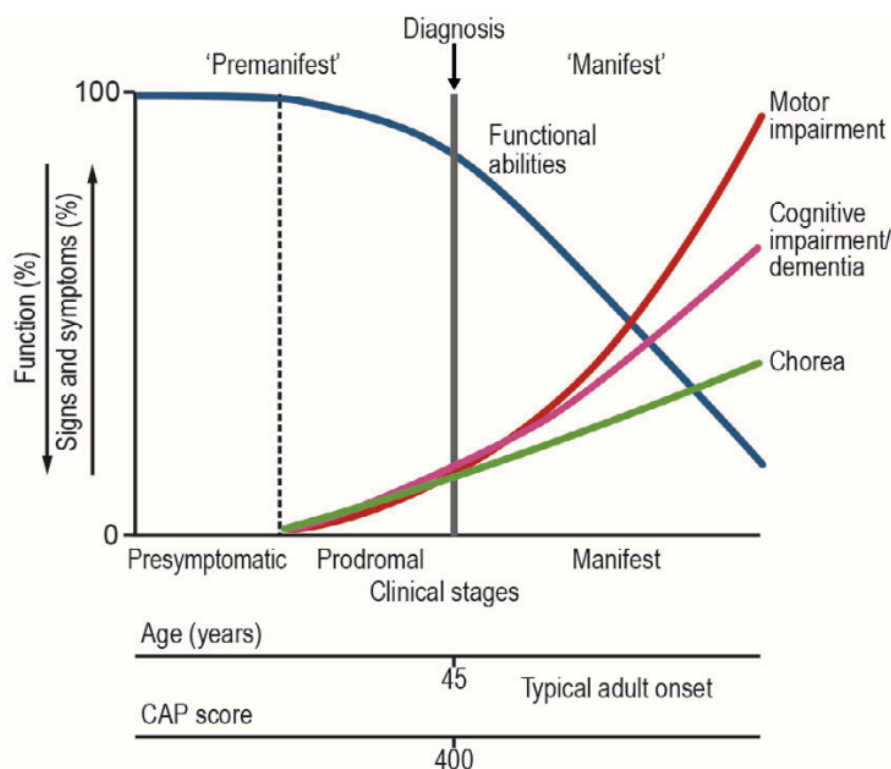
movements are typically the first motor-symptoms reported by patients and family members.²²¹ Early manifestations often include motor restlessness and a decline in fine motor coordination, progressing to choreiform movements over time.²²² As the disease advances, the motor phenotype can evolve to resemble Parkinsonian features, with a reduction in chorea and increased muscular²²³, dysarthria and dysphagia present, with dysphagia the primary cause of hospitalisation in later stages.²²⁴ These phenotypic differences at onset correlate with patterns of cortical atrophy in premotor and supplementary motor areas.²²⁵

Cognitive deficits in HD are similarly heterogeneous but often include slowed processing speed, social cognitive difficulties and executive dysfunction with memory and language functions being impaired later in the disease course.^{226,227,228} Apathy is a highly prevalent behavioural feature, as are impulsivity and irritability.^{229,230} PwHD (denotes a confirmed HD gene-carrier at any disease-stage) are four times as likely to attempt suicide compared to the general population^{231,232} with depression and irritability affecting up to 50% of patients.²³³ Less common symptoms include aggression and psychosis.²²⁶

1.6.2 HD clinical onset

Clinical diagnosis of manifest HD (manHD) is made by a neurologist at the presentation of an unequivocal HD-related, extrapyramidal movement disorder, typically chorea, in individuals who show evidence of deterioration over time, a positive family history and/or positive clinical genetic testing.^{223,234} The age of onset (AoO) of HD is also heterogeneous, ranging from juvenile forms which manifest in the first and second decades of life, to those that receive clinical diagnosis in their 10th decade. The average AoO is between 40 and 50 years of age, with a typical disease duration from clinical diagnosis of 15-20 years.^{222,226,235} A positive genetic test confirming a pathogenic CAG repeat expansion on *HTT* establishes premanifest status in an asymptomatic, unaffected individual. Neurodegeneration and symptom onset are gradual, unfolding over many years. Subtle motor, cognitive, or psychiatric changes are often detectable during the prodromal phase, and can emerge up to 15 years before motor-symptom onset and a formal clinical diagnosis (**Figure 10**).^{223,228,235,236}

Figure 10: Disease stages and trajectories of clinical features of HD. From Ross et al., 2019.²³⁷



1.6.3 Clinical genetics of HD

HD is caused by an unstable trinucleotide (CAG) repeat expansion within exon 1 of the huntingtin (*HTT*) gene. *HTT* was identified in 1993 on the short arm of chromosome 4p16.3.²³⁸ This discovery followed earlier landmark studies in 1983 and 1987 that laid the groundwork for localising the gene and understanding its inheritance pattern.²³⁹ In the general population, individuals typically carry fewer than 36 CAG repeats and do not develop the disease. Expansions of 36–39 repeats show reduced penetrance, while expansions of ≥ 40 repeats are fully penetrant and confer near-certain development of HD, assuming a sufficient lifespan.²¹⁸ HD follows an autosomal dominant pattern of inheritance. Each child of an affected individual has a 50% chance of inheriting the expanded allele.²⁴⁰ Rates of presymptomatic testing for *HTT* in those at risk (50% chance) in Northern Ireland are estimated to be as low as 14.7%²⁴¹, which likely reflects similar rates in the Republic of Ireland.

Rare cases of de novo mutations have been documented in the literature, but the vast majority of cases are familial.^{242–244} These typically arise through mechanisms such as genetic anticipation, whereby repeat lengths increase across successive generations, particularly through paternal transmission.²⁴⁰

1.6.4 Epidemiology of HD

Despite its rarity, HD is one of the most prevalent genetic disorders, present across ethnicities, although penetrance estimates vary across populations and geographic regions.²⁴⁵ Incidence of HD in Ireland is thought to be equivalent to most populations of European origin²⁴⁶, and estimated based on a 1995 study²⁴⁷ in Northern Ireland, which showed a prevalence rate of 6.4 per 100,000 in 1991. This was updated in 2010 to 10.6 per 100,000²⁴¹, citing increased life expectancy for the jump. Both estimates are higher than in North American and European populations, approximately 5.7 per 100,000.²⁴⁸ Studies in Asian populations show much smaller prevalence estimates, 0.4 per 100,000.^{245,248}

1.6.5 Pathophysiology of HD

HD is characterised by widespread neurodegeneration throughout the CNS. Post-mortem studies report an average 19% reduction in total brain volume in affected individuals.²⁴⁹ Wild-type HTT protein is ubiquitously expressed, fundamental for neuronal development in particular.^{218,250} The production of mutant-HTT (mHTT), which occurs in HD, interferes with this process, likely through a loss-of-function mechanism. However, production of this aberrant protein also produces toxic N-terminal fragments, which misfold.^{250,251} Continued deposition of these misfolded proteins form nuclear inclusions which interfere with essential cellular functions, and give characteristic pathological signature of HD. This gain-of-function mechanism causes progressive but wide-spread neuronal cell death. GABAergic medium spiny neurons (MSNs) in the striatum are most sensitive to the cascade of molecular and cellular disturbances that ultimately lead to progressive neurodegeneration,^{218,251} where up to 95% of striatal MSNs are lost.²⁵² This degeneration results in up to 50% volume loss in the dorsal striatum (caudate nucleus and putamen).^{252,253} Regional degeneration is strongly associated with motor impairment.²⁵⁴⁻²⁵⁶ In addition to the striatum, other diffuse brain regions are also affected, including the cerebral cortex^{249,257} and hypothalamus.²⁵⁸⁻²⁶⁰

A vicious cycle of loss-of-function and gain-of-function results in whole brain atrophy and the terminal system-wide impairments seen in late stage HD. Neuronal dysfunction precedes cell death, and pathological changes begin years before the onset of overt clinical symptoms.²²³ Neuroimaging and neuropathological studies have demonstrated that brain atrophy begins long before the clinical onset of symptoms.^{234,256,261-267} This gives rise to a clinical prodromal phase (**Figure 10**), where dysfunction creates progressive sub-clinical phenotypes. This phase presents an

opportunity for early intervention, targeting potentially reversible dysfunction rather than irreversible cell loss.^{218,250}

Ubiquitous expression of mHTT occurs across all cell types and tissues.²⁶⁸ Although HD is usually considered as limited to the CNS, a spectrum of peripheral abnormalities also occur, with evidence of pathology in peripheral systems supporting its classification as a multisystem disorder.²⁶⁹ Significant skeletal muscle atrophy has been observed in both individuals with HD and transgenic mouse models.²⁶⁹ These findings are of particular clinical importance, as cardiac failure is the second most frequent cause of death in HD after pneumonia.²⁷⁰ Recent work suggests that pathology in HD is the result of DNA mediated effects, as opposed to protein misfolding and deposition.²⁷¹ In this case, basal CAG expansion is related to onset and prognosis only in so much as it reduces the steps, or DNA replication errors, needed for individual cells to begin to die. It has challenged the prevailing view of repeat-expansion disorders being the result of gain/loss of protein function.²⁷²

1.6.6 Treatments for HD

The number and variation in symptoms of HD make it a difficult disorder to treat, and despite the genetic aetiology being well understood, there are no approved disease-modifying therapies at present. The EHDN guidelines task force produced standardised clinical practice guidelines for HD symptom management across specialties through a Delphi consensus approach and systematic review of treatment literature.²⁷³

1.6.6.1 Pharmacological therapy

1.6.6.1.1 Symptom management therapies

Pharmacological interventions aimed at symptomatic management of motor disturbances and psychiatric symptoms aim to improve quality of life PwHD.²⁷³ Tetrabenazine is the only drug with randomised-control trial (RCT) support for chorea. It is widely prescribed as a symptomatic treatment.²⁷⁴ Antipsychotics are frequently used off-label to manage motor and behavioural symptoms simultaneously, especially where weight gain (Olanzapine) may be beneficial. Psychiatric symptoms are common at every stage of HD from depression and apathy to anxiety disorders and psychotic symptoms. Therefore treatment with a range of anti-depressants, anxiolytics and antipsychotic medication is common.²¹⁸

There are no effective treatments for cognitive symptoms of HD. Trials of memantine, typically given in Alzheimer's disease, have not shown efficacy.²⁷⁵ Non-

pharmacological behavioural and social interventions are also proposed for milder behavioural presentations and have shown similar efficacy to medication in some studies.²⁷⁶

1.6.6.1.2 Genetic therapies

HD is uniquely placed as a model disorder with a single genetic aetiology to benefit from genetic therapies which neutralise this genetic cause. The advent of genetic therapies has produced a number of clinical trials attempting to do this. uniQure's gene therapy 'AMT-130' and Roche/Spark's new 'RG6662' have entered early-phase clinical trials with encouraging safety signals. Meanwhile, HTT-lowering strategies, ranging from ASOs (e.g.: Tominersen currently in Phase II/III) to small-molecule splice modulators like 'PTC-518', are advancing and showing promising huntingtin reduction and favourable safety results in mid-stage trials.^{277,278} To date, none have been approved.

1.6.7 Phenotypic characteristics & clinical diagnosis of HD

The length of the CAG-repeat expansion correlates strongly with AoO of motor symptoms and disease severity. Increasing CAG-repeat length inversely relates to lower AoO. CAG-repeat length explains 50-70% in the variance in AoO.^{71,279,280} The remaining, as of yet unexplained variance in AoO has high heritability, estimated at about 0.5.²⁸¹ Despite the clear association between repeat length and disease onset, substantial phenotypic variability exists. AoO, clinical profile, progression rate, and severity vary widely even among individuals with similar repeat lengths, suggesting that HD is not strictly monogenic and linear in its expression.²¹⁹ Modifying effects of other genetic variants and environmental exposures likely contribute to this heterogeneity.

It is well established that AoO is also significantly influenced by non-CAG related factors.²⁸² Environment and family-specific factors account for between approximately 10-19% of the variance²⁸³, whilst non-CAG genetic factors, such as *MSH3* and *FAN1* modifier genes, can account for more.^{284,285} Many groups are investigating the influence of genetic modifiers on phenotypic presentation, which are mostly involved in somatic instability of the trinucleotide repeat expansion.^{286,287}

1.6.7.1 HD clinical diagnosis

HD is a clinically heterogeneous disorder, but current diagnostic criteria are based on the emergence of unequivocal signs of HD-characteristic-motor symptoms, often in the presence of the genetic expansion. Neurological examination determines whether

extrapyramidal motor symptoms are present. The Unified HD Rating Scale (UHDRS) has multiple domains of assessment, which include an assessment of motor function. This scale yields a Total Motor Score (TMS) between 0 and 124 as a measure of severity of motor symptoms.²⁸⁸ Furthermore, in individuals with a family history of HD and/or PwHD (premanifest or manHD), the Diagnostic Confidence Level (DCL) assigns a number between 0 and 4 as a crude measure of certainty that extrapyramidal motor disorder is present (**Table 3**). The probability thresholds provide a guide which have been widely adopted clinically and in large scale studies.

Table 3: Diagnostic Confidence Level (DCL) framework for clinician ratings of HD-related motor symptoms.

DCL	Clinical impression
0	Normal
1	Non-specific motor abnormalities
2	Possible onset – 50% confidence symptoms relate to onset of HD
3	Probable onset – 90% confidence symptoms relate to onset of HD
4	Definite onset – 99% confidence symptoms relate to onset of HD

1.6.7.2 HD motor-staging criteria

Functional status and symptom burden are commonly tracked in manifest stages using the UHDRS²⁸⁸ and Total Functional Capacity (TFC) score²⁸⁹, as measures which divide up manHD into various stages. Initial staging systems, such as the Shoulson-Fahn staging are purely descriptive (**Figure 11**).²⁹⁰ Clear biological stages are not present in HD as they are in cancer, for example. Instead staging has been guided by function, often based somewhat arbitrary cut-offs on the DCL, UHDRS scales and TFC which provide pragmatic benefits.^{223,279,290}

After clinical diagnosis, individuals with early stage HD are usually still active and may continue working or driving.²²³ The moderate stage is characterised by a loss of capacity for complex tasks, such as employment, and driving. In the late stage, increasing functional decline results in the need for assistance with activities of daily living.²²³ Although this system is still used for clinical staging, research staging criteria have improved in their specificity, which will be discussed in subsequent sections.²⁵⁶

1.7 HD cognitive/behavioural diagnostic criteria

Cognitive and psychiatric symptoms develop in parallel and often precede the motor symptoms of HD, causing significant impact on quality of life (QoL) prior and subsequent to diagnosis.²⁹¹⁻²⁹⁵ The UHDRS-TMS and DCL are the only measures of diagnostic certainty in HD. Clinical diagnostic criteria which go beyond presentation of motor-symptoms are still not widely accepted. Inclusion of cognitive criteria is more difficult due to the normal variation in cognitive function in the general population.^{296,297} However, many believe motor-only diagnostic criteria misrepresents the complex presentation of early HD. Witjes-Ane et al.²⁹⁸ comment: “*patients and families often retrospectively report the subtle appearance of cognitive impairment and personality changes as heralding disease onset*” (p. 310). Despite recognition of the symptomatic heterogeneity and the functional impairment changes to cognition and behaviour can cause in HD, debate remains around extramotor diagnostic criteria.

Ross et al.²³⁷, along with the Movement Disorder Task Force proposed combined the DCL for motor symptoms, with DSM-5 criteria for the diagnosis of Mild and Major Neurocognitive Disorder (**Table 4**) proposing a standardisation of non-motor characterisation. In addition to motor assessment, these criteria require clinical report or evidence on formal neuropsychological testing of cognitive decline from previous levels of functioning, in one or more domain, which interferes with daily function. The Montreal Cognitive Assessment (MoCA), Trail Making Test, Symbol Digit Modalities Test and others are recommended.²³⁷

Table 4: Diagnostic criteria proposed by Ross et al.²³⁷ based on existing DCL motor confidence criteria with the addition of cognitive criteria.

Diagnosis Stage	Motor Diagnosis Confidence (DCL)	Cognitive Status
(1) Presymptomatic HD	Dx conf 0 or 1	Normal
(2-A) Prodromal HD (A)	Dx conf 2	Minor or major neurocognitive changes
(2-B) Prodromal HD (B)	Dx conf 3	Normal (unchanged) cognition
(3-A) ManHD (A)	Dx conf 3	Minor or major neurocognitive changes
(3-B) ManHD (B)	Dx conf 4	Normal (unchanged) cognition

'A' denotes cognitive change present at various levels of motor impairment. 'B' denotes normal cognition.

1.7.1.1 MCI-HD

Duff et al.(2010)²⁹⁹ and later Julayanont et al.(2020)³⁰⁰ proposed an extramotor classification system for mild cognitive impairment (MCI) in HD using a domain-based approach with executive dysfunction as the core tenet. Applying these criteria to the ENROLL-HD database, they found 84% and 5% of PwHD met criteria for MCI and dementia respectively at clinical diagnosis. Five years later, the proportion with MCI was 24%, whereas 69% met diagnostic criteria for dementia. Biglan et al. in the PREDICT-HD study³⁰¹ compared motor-based diagnosis with a multidimensional approach that incorporated non-motor cognitive/behavioural features. Using the existing cognitive and behavioural components from the UHDRS, they uncovered three clusters of patient phenotypes at diagnosis: primarily cognitively impaired, behaviourally impaired, and cognitively preserved.^{299,301} Expectedly, a multidimensional diagnosis determined an earlier AoO for many PwHD with milder motor and cognitive symptoms and captured those who had primarily non-motor onset phenotypes. Garcia-Gorro et al.³⁰² found evidence for a distinction of psychiatric symptom onset (psychiatric-predominant), but only concluded that the second cluster at diagnosis was a motor-cognitive predominant phenotype, which progressed together. They described shared neuroanatomical substrates for cognitive- and motor-symptom progression, with distinct circuit pathology producing a psychiatric phenotype.

Psychiatric conditions such as depression, psychosis, and suicide are overrepresented in the family histories of individuals with HD, likely reflecting early and variable neuropsychiatric manifestations of the gene expansion.^{233,303,304} Although acknowledging the prevalence of extramotor symptoms in prodromal and early HD, Ross et al.²³⁷ caution against the inclusion of behavioural features in diagnostic and staging frameworks (**Table 4**). They cite the commonality of psychiatric symptoms in the general population, which may be even more common in at-risk individuals due to the psychological impact HD can have on families, and not necessarily organic disease-related pathology. Instead, they conclude that the presentation of these “emotional aspects” warrant close observation of cognitive and motor symptomatology, but should not be considered in diagnostic criteria.

1.7.1.2 Research staging criteria: HD

Research staging criteria were subsequently published by Tabrizi et al.^{256,305}, known as the HD-Integrated Staging System (HD-ISS; **Figure 11**). These criteria aim to move research and clinical trials towards biological classification based on measurable

indicators of underlying pathophysiology, as well as clinical phenotype. It aimed to replace older staging terminology based on function alone. Widely accepted, it is restricted to use for research and clinical trials, and as with other research staging criteria³⁰⁶, it does not replace clinical judgment in diagnosing HD.

1.7.1.3 Seeking consensus for non-motor HD diagnosis

Extramotor symptoms are not yet recognised by formal clinical diagnostic criteria. This practice is coming under increasing pressure for revision as the prominence of extramotor symptoms are increasingly recognised in premanifest and prodromal stages^{237,307,308}, and in some cases are a better reflection of disease progression.^{228,309} Perspectives from Considine et al.^{307,308} proposed a revision of clinical diagnostic criteria, and that cognitive and/or behavioural symptoms be sufficient for diagnosing clinical onset and symptomatic HD. Rather than confining these individuals to a premanifest or prodromal categorisation due to the absence of unequivocal motor signs ($DCL < 4$), the presence of a known CAG repeat expansion in addition to new onset cognitive/behavioural symptoms can be diagnostic of clinical onset, at varying degrees of probability: Possible and Probable symptomatic HD (**Appendix 1**). The Huntington Study Group (HSG) Neuropsychology Working Group recently published a position paper, highlighting the tasks which should be included in the neuropsychological assessment of HD.³¹⁰

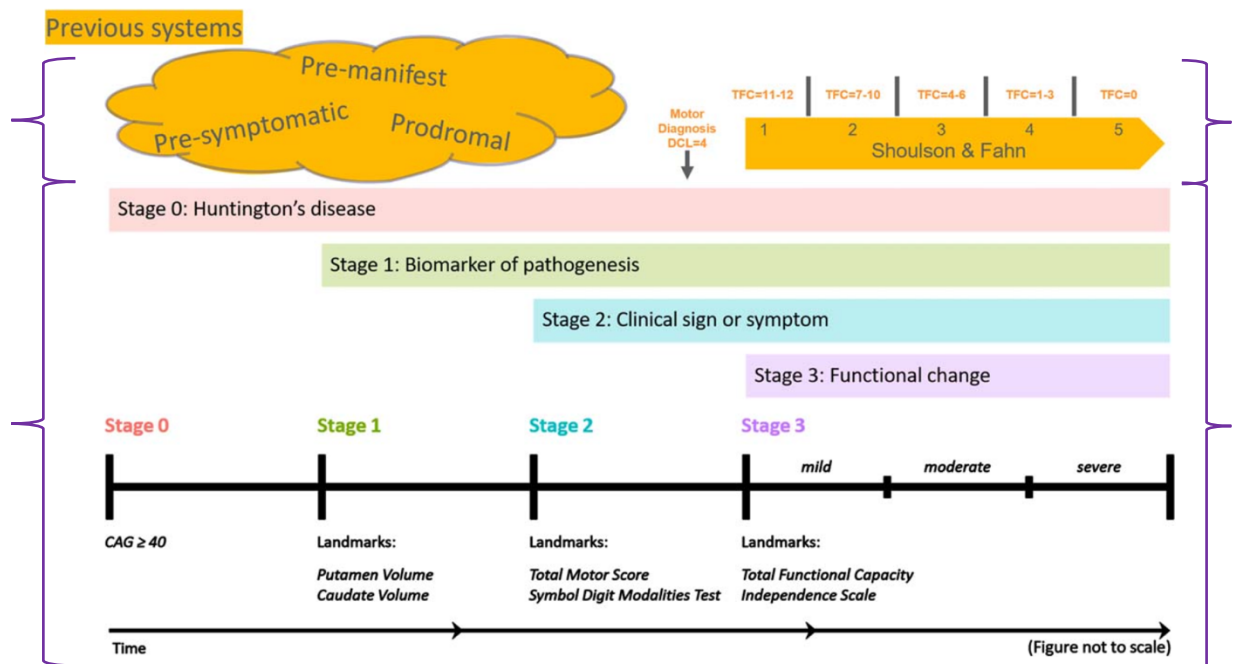


Figure 11: HD-ISS and Shoulson and Fahn Staging Systems.

Top: Previous staging systems for HD; Bottom: The HD-ISS. From Tabrizi et al.²⁵⁶ and Sampaio et al.³¹¹

1.7.2 Prediction models of onset & progression in HD

The strong association between CAG repeat number and age at onset has been leveraged to make predictions about the age a person with an expanded CAG allele will develop the (motor) symptoms of HD (AoO). This genotype-phenotype correlation has led to the development of several predictive models aimed at estimating cumulative disease burden³¹², probability of clinical diagnosis⁷¹, and estimates of disease progression.³¹³⁻³¹⁵

The first of these was proposed by Penney et al.³¹², who termed their estimate, ‘disease burden’ following the association between CAG repeat number, age and striatal pathology at autopsy. The disease burden score has become a widely used proxy for lifetime exposure to mHTT throughout the disease course.^{261,316} Estimated progression of striatal pathology was a function of time and CAG repeat length and took the form of:

$$\text{‘Disease Burden’} = \text{AGE} \times (\text{CAG} - L)$$

where L = a constant.

To improve clarity and standardisation, this metric has been referred to more objectively as the CAG-Age Product (CAP) score. The constant L represents the lowest CAG repeat length at which HD-related neuropathology is likely to occur; its value can vary slightly depending on the model, with Zhang et al.³¹⁷ setting $L = 33.66$ based on observed data from the PREDICT-HD study.³¹⁷

To enhance clinical and research utility, Zhang et al.³¹⁷ further scaled the CAP score to generate the CAP_s metric, which expresses disease burden relative to the age at which there is a 50% probability of motor diagnosis within five years. This allowed classification of premanifest PwHD into Low, Medium, and High disease burden groups, using ROC-derived cut-offs.

Ross et al.²²³ and Warner et al.^{315,318} proposed an alternative standardisation, known as the CAP100 score, which anchors the scale such that a score of 100 corresponds to the estimated age of clinical motor onset.^{223,315} The CAP100 is particularly useful in imaging and progression models, as it aligns age and CAG length to a biologically meaningful transition point and provides an intuitively interpretable index of disease progression in premanifest individuals.³¹⁸

Langbehn et al.⁷¹ created a parametric survival model which not only include CAG repeat number and a person’s age, but also modelled the variation in onset not attributable to CAG repeat length. They applied survival analysis techniques to account for both

diagnosed and undiagnosed individuals. This flexibility allowed more accurate and unbiased age-conditional risk predictions at different ages and CAG repeat lengths, and thus, predictions about motor-symptom onset for premanifest individuals. It also allows estimation of CAG-specific penetrance rates for lower number of repeats. It has high accuracy for CAG repeats between 36 to 56, with reduced accuracy for those between 36 and 41. The Langbehn et al. parametric survival model is the most robust predictive model using only CAG and age.^{71,314}

Other predictive models include the Prognostic index for HD (PI_{HD})³¹³ and the normalised version, PIN_{HD} .³¹⁹ Calculation integrates age, CAG repeat length, total motor score (UHDRS-TMS), and performance on the Symbol Digit Modalities Test (SDMT; test of psychomotor processing speed) to estimate proximity to motor diagnosis. These indices focus on rate of symptom progression and have demonstrated strong predictive validity across multiple independent observational cohorts. PIN_{HD} was introduced to enhance interpretability and facilitate clinical trial design.³¹⁹ Thresholds allow for enrichment of clinical trial samples with individuals at greater risk of disease progression and closer to estimated disease manifestation, thereby improving statistical power and reducing required sample sizes.^{256,320}

These CAG-based estimates are helpful, but are not based on objective measures within individuals. Analysing the large PREDICT-HD dataset, Stout et al.(2011)²²⁸ found that cognitive assessment accounted for a third of the variance in estimated disease onset, with three times the predictive ability of motor function, underscoring the relevance of cognitive function for prognostication.

1.7.2.1 HD progression sub-types

Markers of progression phenotypes have been explored extensively in HD. The TRACK-HD study is the most comprehensive study of symptom trajectories in HD, assessing a wide range of biomarkers over a three year period in a large, well characterised sample²⁵⁵, including neuroimaging markers, clinical and psychiatric assessments, cognitive performance and quantitative motor tasks. They report striatal volume as the strongest marker of progression, later shown to have high accuracy in differentiating moderate and fast progressors.³²⁰

1.7.2.1.1 Natural history studies

Dorsey et al.³²¹ analysed data from participants with at least 3 consecutive years of longitudinal data in the Cooperative Huntington's Observational Research Trial (COHORT). Aiming to outline the natural history of HD, they trajectories of motor, cognitive,

behavioural, and functional outcomes. They concluded that manHD participants displayed a steady decline in function across all cardinal symptoms, regardless of baseline functional status or disease burden level.³²¹ Contrasting to many studies which found domain specific and variable rates of decline across the disease timeline. Research has attempted to create symptom based staging systems and more objective measures of clinical disease progression. But heterogeneous symptom and progression profiles has meant this method did not prove useful.²²¹

1.7.3 Neuroimaging HD

In HD, MRI studies show degeneration of the caudate and putamen, causing dysfunction in the pathways governing higher-order cognition in the frontal lobes. Early and progressive cognitive alterations in PwHD are highly correlated with volumetric reduction and morphological shape differences in the striatum, with performance on a task of executive function most strongly associated with alterations of the left putamen.³²² Structural and functional neuroimaging studies show patterns of brain atrophy that underlies clinical heterogeneity in HD, with regional atrophy related to cognitive, motor and psychiatric outcomes. Rosas et al.^{253,257} conducted two small neuroimaging studies in early HD. They showed diffuse cortical degeneration is linked to cognitive/psychiatric-predominant presentations, whereas more restricted atrophy (confined to striatum) yields primarily motor symptoms.

1.7.4 The function of the striatum

The dorsal striatum, comprising the caudate nucleus and putamen, together with the nucleus accumbens (ventral striatum), forms the major input region of the basal ganglia, which is central to motor and reward-related processing.³²³ Functionally, the striatum acts as an inhibitory control hub within cortico-basal ganglia circuits, playing a critical role in the coordination and execution of motor programmes, as well as in modulating cognitive and affective processes. The dominant neuronal population in the striatum consists of GABAergic MSNs, which integrate excitatory glutamatergic inputs from the cortex and thalamus and transmit inhibitory output to downstream basal ganglia nuclei.³²⁴⁻³²⁷ This inhibitory signalling is essential for suppressing competing or inappropriate motor programmes, thereby enabling the selection and execution of goal-directed behaviour.

In HD, progressive degeneration of striatal MSNs disrupts the structural and functional integrity of fronto-striatal circuits. These networks link the striatum with regions of the frontal cortex. Disruption results in the characteristic hyperkinetic motor symptoms, typically chorea, due to impaired inhibitory control within these circuits. Importantly, the impact of striatal degeneration extends beyond motor systems to include associative (cognitive) and limbic (affective) pathways^{323,328,329} contributing to the cognitive and behavioural disturbances observed in HD (**Figure 12**).^{330,331}

1.7.4.1 Fronto-subcortical circuits

There are three primary prefrontal circuits which govern cognition, behaviour and neuropsychiatry: the dorsolateral prefrontal circuit (involved in the executive functions), the orbitofrontal circuit (responsible for social and emotional regulation), and the anterior cingulate circuit (hub of the salience network governing goal-directed behaviour and motivation). Each circuit originates in a distinct frontal region and projects via the striatum and globus pallidus to the thalamus, before completing a closed-loop back to cortex.^{328,329} This conceptualisation of fronto-subcortical networks offers a unifying framework linking anatomy, neurochemistry, and behavioural dysfunction.^{328,329} Stuss' neuroanatomical models of the executive functions are based on the initial work of Cummings, and forms a theoretical underpinnings for this thesis.^{332,333} They are discussed in the next chapter.

1.8 The neuropsychology of HD

Cognitive and behavioural/psychiatric impairment frequently precede motor symptom presentation in HD, have higher predictive ability than motor scores and often contribute more to caregiver burden than the motor symptoms.^{235,298,334,335} A factor analysis a large sample within the PREDICT-HD based on disclosure of everyday cognitions identified five- and seven-factor structures that provided good explanatory power to the cognitive profile of early/prodromal HD, comprising a global cognitive factor alongside six specific domains (memory, language, visuospatial skills, planning, organisation, and divided attention).³³⁶ Companions cited executive dysfunction (the latter three domains) as the earliest symptom in manHD which deteriorated over time. These symptom reports showed more sensitivity to progression than traditional TFC measures.³³⁶ However, Williams overview did not comprehensively cover the neuropsychological profile of HD, with some key domains omitted. Domains most relevant in HD are addressed here, with the next chapter combining the neuropsychology of both ALS and HD, particularly focusing on early and premanifest stages, to complete the overview.

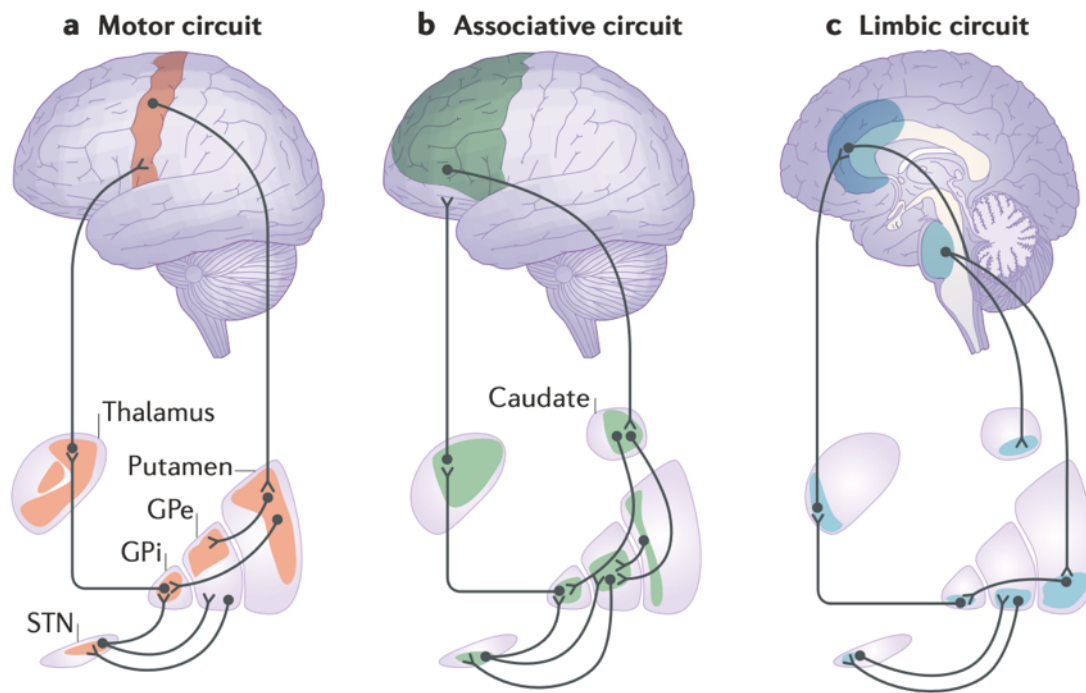


Figure 12: The functional segmentation of the fronto-striatal circuits.

The figure shows the topological organisation of striatal nuclei into motor, associative and limbic regions and their corresponding projections through basal ganglia–thalamo–cortical loops. Although these functional territories are somewhat distinct, the networks overlap and interact to cross-modulate signals from the cortex and other subcortical structures (e.g.: Thalamus, STN). GPe/i = External/Internal segments of the Globus Pallidus; STN = Subthalamic Nucleus. From Jahanshahi et al.³²³

1.8.1 HD: A fronto-striatal syndrome

Fronto-basal ganglia-associated cognitive and behaviour change have been well characterised in HD with executive and social cognitive deficits evident early on³³⁷, though patterns of impairment vary and often also include memory and language impairments as the disease progresses.^{338–340} It shows a progressive fronto-subcortical cognitive dysfunction with sparing of more posterior cortical functions in early disease, with progression to a subcortical dementia.³⁴¹ Executive functions are almost universally and significantly impacted.^{335,342} Even in mild or prodromal HD, patients typically show deficits in tasks requiring planning, cognitive flexibility and divided attention^{343–345} The most prominent and consistent finding is of reduced psychomotor processing speed.³⁴⁶ The SDMT is a sensitive but non-specific task of general cerebral dysfunction commonly used in HD and other disorders, such as Multiple Sclerosis (MS).³⁴⁷

A study by Ho et al.³⁴¹ assessed 61 early-stage manifest PwHD, followed longitudinally for ≥ 3 yearly assessments. They found a selective and progressive decline in attention and executive tasks (digit symbol substitution, Stroop task, and verbal fluency),

particularly those with a timed component, but a relative sparing of general cognition (e.g. Mini-Mental State Exam), semantic memory, and delayed memory recall. Anosognosia or poor insight into cognitive function is also present in HD. Another study by Ho et al.(2006)³⁴⁸ found impaired executive function in PwHD, which the patients underestimated consistently by 26%, despite accurately identifying the function of their caregivers.

1.8.2 Social cognition in HD

Social cognition is a broad cognitive domain concerning all mental activities relating to the interpretation and action in a social context. It is defined as the ability to recognise the thoughts, intentions and emotions of others in a social context.^{180,349} This can include affect recognition, moral judgement, social perception, empathy and theory of mind (ToM).^{127,350-353} Interpersonal difficulties, personality changes and other impairments of social functioning are cited by many as the first indicators of disease onset in HD.^{354,355}

Emotion, or affect recognition is the most replicated, most common and earliest cognitive difficulty detectable in preHD.^{356,357} Eddy et al.³⁵⁶ found that facial-affect recognition is impaired at the group level, up to 15 years before the estimated onset of motor symptoms. Difficulties appear to disproportionately affect negative emotions.³⁵⁸ Snowden et al.³⁵⁹ demonstrate a consistent multimodal impairment for anger, fear, and disgust in PwHD, compared to non-specific deficits in FTD.

1.8.2.1 Theory of mind

ToM is higher-order cognitive domain within social cognition which relates to a person's ability to infer the mental state of another or others.^{127,360} It further is divided into cognitive and affective theory of mind. Cognitive theory of mind concerns interpreting the thoughts, motives, beliefs and intentions of another person. Affective theory of mind relates to the ability to understand the affective state, or emotions of another.³⁶¹⁻³⁶³ A meta-analysis by Bora et al.³⁵⁸ reported evidence for ToM and emotion recognition deficits in HD, which demonstrated progression in some studies. Effects were evident in manHD, and strongest for complex ToM tasks. Escudero-Cabarcasa et al.³⁶⁴ carried out a systematic review of 27 studies over 20 years of ToM research in HD. They report that affective ToM is a good candidate biomarker for early dysfunction, commonly assessed by The Reading the mind in the Eyes Test (RMET). preHD participants have demonstrated impairment on the RMET, preceding marked motor impairment.³⁶⁵

Along with Bora et al.³⁵⁸, Escudero-Cabarcasa et al.³⁶⁴ recommend that social cognitive function should form a key part of the assessment of HD. Following this research, and many similar calls for inclusion of social cognitive function in the cognitive assessment of HD, the Neuropsychology Working Group of the HSG have recently recognised social cognition as a key cognitive domain in the diagnosis and clinical trajectory of HD.³¹⁰ They call for a more rigorous neuropsychological assessment as standard in the clinical diagnosis and management of HD, given the impact social cognitive impairments can have on the lives of PwHD.

This expands from HD alone to neurodegenerative disorders in general where cognition is impacted, including Alzheimer's disease (AD), FTD and Parkinson's disease (PD).³⁶⁶ The DSM-V places social cognition as one of six domains on which a diagnosis of major- and mild-Neurocognitive Disorder can be diagnosed. The *SIGNATURE* initiative³⁶⁶ aims to harmonise social cognitive testing in NCDs by clarifying current practices in assessment for relevant conditions. It found that, despite evidence for social cognitive deficits in many NCDs, it remains routinely underassessed in clinical settings. They expose a critical gap in the diagnostic pipeline where social cognitive impairments are neglected despite their clinical relevance and impact on patient and caregiver wellbeing.³⁶⁷⁻³⁷¹

1.8.2.2 Other domains of social cognition

Empathy, social-conceptual knowledge, moral reasoning, are other domains of social cognitive function that have shown some signs of dysfunction in HD and related disorders that have been linked to alterations to overlapping but distinct neural networks.^{133,372}

1.8.2.3 Neural correlates of social cognition

Evidence suggests that social cognitive abilities are mediated by a distributed fronto-striatal network.^{349,373} Behavioural symptoms frequently observed in neurodegenerative disorders, such as apathy, reduced empathy, and violations of social norms show functional and structural overlap with regions also associated with social cognitive dysfunction.^{127,355,373} Performance on the RMET scores have been associated with grey matter volume of the dorsomedial prefrontal cortex, the temporoparietal junction and the precuneus.³⁷⁴ The relationship between brain function and decreased test performance may change throughout the HD disease course. Eddy et al.³⁷⁵ found performance on the RMET was significantly associated with ACC activity in preHD participants, which included executive and emotion perception areas in manHD groups.

Cognitive ToM is associated with right caudate volume in HD, whereas affective ToM correlated with right-sided anterior and posterior insula volumes.³⁶⁵ This distinction for the two facets of ToM, localised to different brain regions, reinforces the multi-component model of social cognition. **Figure 13a** highlights the interconnectedness of social brain networks with areas important for executive control (DLPFC and ACC), cortical and limbic reward centres (insula, OFC and amygdala), and the dorsal and ventral striatum, primary areas of degeneration in HD. **Figure 13b** outlines areas of the brain and associated processes particularly relevant for different aspects of ToM.

1.8.3 Verbal fluency

Verbal fluency is consistently impaired in HD, reflecting progressive frontostriatal dysfunction. Unlike ALS, performance does not differentiate these domains. Both phonemic (letter) and semantic (category) fluency decline, but the impairment is more closely tied to executive rather than lexical-semantic dysfunction.³⁷⁶ Longitudinal studies show that phonemic switching ability, an executive process reliant on DLPFC circuits, underlies deficits in both fluency tasks.¹⁴⁰ Function declines significantly over time, whereas clustering and semantic switching remain comparatively preserved, indicating a selective deficit in cognitive flexibility rather than semantic retrieval across both tasks.

1.8.4 Behavioural features of HD

As well as the behavioural consequences of social cognitive deficits and disinhibition, common behavioural changes in HD are irritability, impulsivity and difficulty with interpersonal relationships. These changes are often associated with dysfunction of orbitofrontal circuitry and caudate atrophy.^{178,323,377} Up to 98% of PwHD will experience neuropsychiatric symptoms during the course of disease³⁰³, often progressing from the premanifest phase.³⁷⁸ Depression is the primary psychiatric symptom and can present up to decades before clinical diagnosis and fluctuate with time.^{379,380} OCD-like symptoms (e.g.: perseverative thinking) are rarer, but not uncommon. Psychosis, occasionally with hallucinations, is also an often disabling but rarer symptom.^{291,304} Apathy is particularly common and tends to worsen with disease progression.³⁸¹

1.8.4.1 Apathy

Apathy is a significant clinical feature of both ALS and HD.^{152,158} In PwHD, rates of apathy in the premanifest phase range from 11-64%, depending on the study.^{304,382-384} Apathy is more prevalent, but equally variable in the manifest stage of HD, with estimates

ranging from 47-76%.^{381,385,386} Symptoms of apathy are predictive of cognitive decline in manHD.^{384,387}

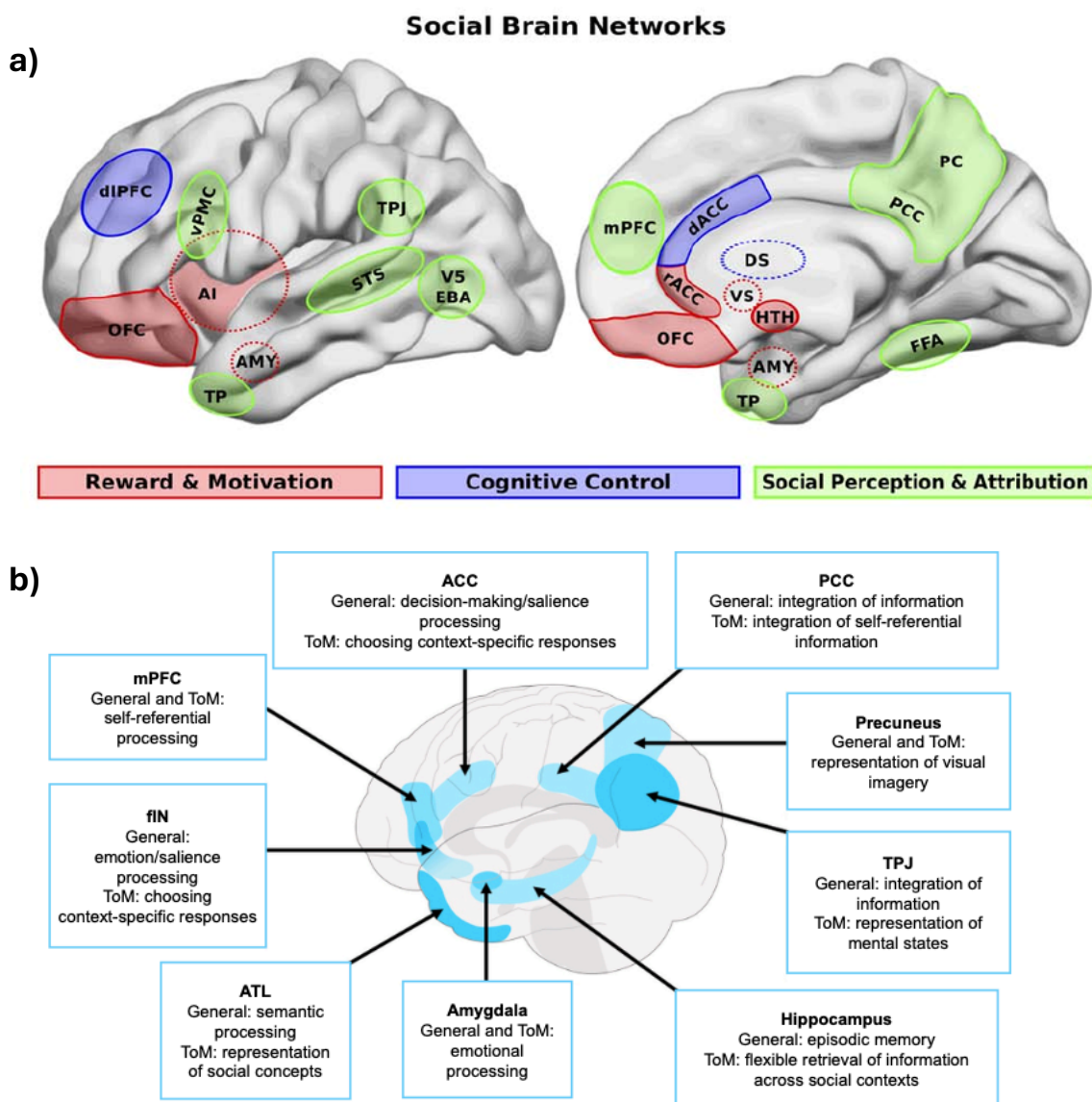


Figure 13: The social cognitive brain.

a) Brain regions involved in social cognitive processing. From Billeke, Boardman, and Doraiswamy³⁸⁸; **b)** Schematic of the regions involved in theory of mind. From Strikwerda, Raman and Irish.¹³³

NOTE: Definitions: ACC – anterior cingulate cortex; AI – anterior insula; AMY – amygdala; ATL – anterior temporal lobe; dACC – dorsal anterior cingulate cortex; dIPFC – dorsolateral prefrontal cortex; DS – dorsal striatum; EBA – extrastriate body area; FFA – fusiform face area; fIN – frontoinsula cortex; HTH – hypothalamus; mPFC – medial prefrontal cortex; PCC – posterior cingulate cortex; PC – precuneus; rACC – rostral anterior cingulate cortex; STS – superior temporal sulcus; TP – temporal pole; TPJ – temporoparietal junction; ToM – theory of mind; vPMC – ventral premotor cortex; VS – ventral striatum.

1.9 Neuropsychological trajectories in HD

Heterogeneity in HD suggests that it is not a uniform clinical entity. Evidence exists for the presence of sub-phenotypes, which are differentially expressed throughout the disease course. The rate of change of cognitive and motor scores are also indicative of progression phenotypes, independent of CAG repeat length and age.^{228,255,389} Progression is not linear however, as rate of change increases over time as premanifest participants approach their predicted AoO.²⁵⁵ Two recent studies in prodromal and early symptomatic HD provide important and complimentary insights into cognitive symptom heterogeneity and progression, using the ENROLL-HD study data.^{389,390} Both elucidate relationships between cognitive function and motor-symptom progression.

Migliore et al.³⁹⁰ retrospectively analysed the cognitive performance of premanifest gene carriers who later phenoconverted (i.e.: transitioned from asymptomatic to the clinical phenotype; preHD to manHD), aiming to investigate whether baseline cognitive composite scores (CCS) could predict motor and behavioural severity following clinical diagnosis. Associations between baseline CCS and subsequent motor and behavioural severity were observed independently of traditional CAG-related predictions. Contrasting but related to this are findings that baseline cognitive function does not have discriminatory power, but it is the slope of change to cognitive scores that predict cognitive as well as motor, psychiatric and functional decline.³⁸⁹ They reported distinct clusters of fast- and slow-progressing groups based on 1-year cognitive-change scores, predicting long term motor, cognitive and psychiatric decline, independent of age, CAG, and disease duration.

Both studies confirm the prognostic relevance of early cognitive changes, but diverge in emphasis. Migliore et al.³⁹⁰ imply that baseline executive dysfunction reflects latent disease processes, predicting disease severity years in advance. Martínez-Horta's results caution against overinterpreting single timepoint assessments and instead advocate for close monitoring of change trajectories. Together, they reinforce a key message: cognitive decline in HD is neither homogeneous nor fully accounted for by CAG-associated factors. If decline in HD is linear across symptom domains as reported by Dorsey et al.³²¹, this would argue against the existence of phenotypic subtypes. However, others have described distinct cognitive-behavioural clusters in early manHD, particularly Martínez-Horta et al.³⁸⁹. Group-level analyses may obscure this variability, as divergent individual trajectories can cancel each other out. As recognition grows for the early emergence of non-motor symptoms, there is an increasing need for more refined sub-

phenotyping approaches to disentangle disease heterogeneity. Neuropsychological assessment plays a central role in the detection, and clinical diagnosis and management of prodromal cognitive dysfunction.

1.10 Conclusion

The first two-part chapter presents a comprehensive overview of the clinical, genetic, and neuropsychological features of ALS and HD. Both are multi-system disorders, impacting diverse cognitive and behavioural networks, with motor-dysfunction as the cardinal feature. The ALS section highlights the presence of cognitive and behavioural symptoms in up to 50% of cases, the influence of *C9orf72* on phenotype and prognosis, and growing evidence for cognitive endophenotypes in unaffected relatives. In HD, early cognitive changes in are prognostically relevant but as in ALS, also heterogeneous. These findings underscore the critical role of neuropsychological assessment, laying the foundation for subsequent chapters, which examine neurocognitive profiles, tools for detection, and the role of endophenotyping in identifying heritable, subclinical markers of disease liability. The next chapter will combine insights from both disorders, to compare and contrast methods and mechanistic understandings with a transdiagnostic neuropsychological approach to the presymptomatic and early symptomatic phases of disease.

2 Chapter 2:

A transdiagnostic approach

2.1 Introduction

Whilst ALS and HD have many distinct features, there are a range of commonalities. Both are heritable disorders, with genetic aetiologies. Progressive motor dysfunction is the hallmark, although cognitive impairment forms a significant portion of the symptomatology and clinical picture. Insidious cognitive decline is a feature in up to 54% of patients with ALS throughout the disease course.² Almost all PwHD experience cognitive impairment by the later manifest disease stages. It is estimated that up to 40% of premanifest PwHD, also experience some form of cognitive decline prior to motor-symptom onset.^{299,335,346,391-393} These ‘extramotor’ symptoms can have significant impact on patient functional ability and caregiver burden.^{368,370,394} Full genetic penetrance of the monogenetic cause of HD (*HTT*) offers a valuable transdiagnostic model for investigating the presymptomatic phase and early therapeutic intervention in neurodegenerative conditions where no definitive preclinical diagnostic tools exist.

This literature review chapter describes the factors shared by at-risk and affected populations in HD and *C9orf72*-ALS kindreds, the conceptual and biological associations between the disorders, and key neuropsychological constructs affected in early, presymptomatic, and at-risk stages. This aims to elucidate lessons from one field of research that may be of relevance to the other, with a transdiagnostic view of early cognitive and behavioural changes in *C9orf72*-related ALS and HD.

2.1.1 Why study ALS & HD together?

A transdiagnostic approach examining overlapping spectra of neuropsychological dysfunction might identify shared early cognitive/behavioural phenotypes and underlying mechanisms and enable a better understanding of the neural networks involved in normal cognitive functioning. It is acknowledged that a combined framework risks oversimplification, and should not preclude the need for precision and disease-specific approaches in clinical care and research. However, the fields have largely evolved

independently. Insights that have not been shared between them may inform best practice in neuropsychological assessment and care, integrated clinical service provision, and the study of presymptomatic phases in genetic disorders. Both disorders share underlying fronto-striatal network dysfunctions which contribute to heterogeneous cognitive and behavioural phenotypes. Their genetic aetiologies share relevance for frameworks in clinical genetics, and as the fields advance toward genetic disease-modifying therapies, examining therapeutic advances and challenges in one disorder can offer valuable translational insights for the other. Such cross-disorder learning may improve clinical trial design, guide biomarker selection, and optimise intervention strategies by leveraging both shared and disorder-specific mechanisms

The convergence of cognitive network impairment in ALS and HD supports the value of examining shared mechanisms across disorders, particularly in the context of neuropsychological assessment and longitudinal clinical trials where repeated, standardised evaluations are essential.^{391,395-397} From a neuropsychological perspective, comparing distinct but overlapping syndromes with similar extramotor features and identifiable presymptomatic phases offers a unique opportunity to disentangle disorder-specific markers from transdiagnostic features of neurodegeneration affecting frontal networks. This has both theoretical and clinical significance, especially when considering phenotypic variability, cognitive endophenotypes, and treatment response.

This thesis takes a transdiagnostic, domain-based approach to cognitive/behavioural symptom evaluation, building on extensive past work in our group. These methods are applied to HD and *C9orf72*-ALS, contributing to the growing presymptomatic literature, and the movement towards non-motor diagnostic criteria for HD.^{307,308}

2.2 Phenotypic heterogeneity & cognitive-behavioural subtypes

Both ALS and HD display significant variability in their clinical presentations, from age of onset, motor symptom profile, cognitive/behavioural features and prognosis. The study of at-risk gene-carriers and patients early in the disease course as they approach diagnosis can reveal early prognostic indicators. Broad phenotypic characterisation of disease through eponymous classification based on clinical presentation continues to be useful, but it also becoming insufficient in the prognostic determination and treatment of disease. With the advent of genetic therapies and precision medicine, clinical research increasingly aims to disambiguate clinical heterogeneity by identifying clusters of

diagnostic sub-categories through a deep phenotyping approach.^{16,183,237,307} The examination of cognition and cognitive correlates is an important part of this sub-classification, supporting clinical prognostication and higher powered clinical trials through homogeneous sampling.

2.2.1 Heterogeneity of cognitive & behavioural symptom development

Variability in the presentation of cognitive and behavioural symptoms in ALS might be expected, given the varied genetic and pathophysiological causes. In *C9orf72* kindreds, where psychiatric and neurological disease is more common in the family history due to gene pleiotropy^{190,191}, there still exists significant variability in site of onset, disease progression and presence of cognitive symptoms.^{56,64,68,398} This suggests C9-ALS is a distinct form of ALS, but itself is highly heterogeneous. HD is monogenetic, and so, the distal genotype is diagnostic. Fully penetrant *HTT* is a direct indicator of disease. But CAG-repeat length does not account for total variability in clinical severity, rate of progression, or symptomology in HD. The remainder (~20-30%) is largely unexplained.^{71,219}

Incorporating the concept of endophenotypes, introduced in Chapter 1, into ALS research may also prompt a shift in how the disease is conceptualised. Classification systems may instead reframe disorders as constellations of co-occurring endophenotypic traits, each linked to distinct genetic mechanisms, particularly useful in polygenic and oligogenic disorders, such as ALS.⁵⁹ This could pave the way for more targeted and personalised therapeutic strategies, directed at specific trait profiles rather than broad disease categories. This approach also reflects a broader movement in neuropsychiatry towards dimensional frameworks. The Research Domain Criteria (RDoC)³⁹⁹ prioritises mechanisms and domains over traditional diagnostic labels in the Diagnostic and Statistics Manual, typically for psychiatric disease, but also includes major and mild neurocognitive disorders. This too may be of benefit to classification of heterogeneous neurodegenerative disorders of the motor system.⁴⁰⁰⁻⁴⁰⁴ Within each of the six domains of the RDoC framework are specific constructs that can be studied across multiple levels of analysis, from molecular and genetic markers to brain circuitry, physiological responses, behaviour, and self-report measures. Its primary aim is to identify the biological, psychological, and behavioural pathways that underlie vulnerability and resilience to psychiatric conditions, not dissimilar to the endophenotype approach.³⁹⁹ Conceptualising HD as such may have benefits for clinical management, prognosis and research/trial stratification, but, as a monogenetic disorder of

large effect, it is unlikely to benefit genetic studies as it would in ALS, and has done in psychiatry.⁴⁰⁵

2.2.2 Extramotor dysfunction caused by frontal-subcortical circuit degeneration

The extramotor symptomatology observed in HD and ALS is due to neuronal cell loss, primarily in the striatum for HD and in fronto-temporal regions and the basal ganglia in ALS-FTSD, impacting fronto-striatal and other frontal-subcortical network function²⁵¹, and downstream frontal lobe function early in the disease. The most extensively studied cognitive domains in HD and ALS are the executive functions. Executive processes rely predominantly, although not exclusively, on the prefrontal and frontal cortices and the network of connecting sub-cortical circuits, with evidence coming from neuroimaging and lesion studies to support this.³²⁹ Fronto-striatal network disruption results in similar, but distinct patterns of cognitive and behavioural deficits across the disorders network disruption results in similar, but distinct patterns of cognitive and behavioural deficits due to a breakdown of the multiple parallel and independent networks. This network may be conceptualised by neuroanatomical models provided by Stuss (discussed at the end of this chapter; **Figure 16**)³³² and Jahanshahi et al. (2015).³²³ Multiple fronto-striatal circuits are involved in successful completion of executive tasks.⁴⁰⁶ Numerous models have been proposed to explain the relationships between these circuits and the underlying cognitive/behavioural processes, the most widely known of which are integral to the functions of the Supervisory Attentional System (SAS) or Central Executive models proposed by Norman and Shallice¹²³ and Baddeley¹²⁴ respectively. Isolated impairment of these functions can indicate dysfunction in a particular circuit, using neuropsychological techniques and these models.^{323,332} A transdiagnostic approach enables the identification of shared and distinct cognitive-behavioural phenotypes.

2.2.2.1 Commonalities in the neuropsychological disorder profile

Fronto-basal ganglia-associated cognitive and behaviour change are well documented in HD and ALS.⁴⁰⁷ Both show a spectrum of cognitive/behavioural impairments—particularly executive dysfunction, apathy, and social cognitive deficits^{170,337,408}, but the specific patterns of impairment vary between and within diagnoses.^{338-340,409-412} Executive dysfunction is diagnostic of ALSci, particularly phonemic verbal fluency, with language and social cognition also affected.¹⁶ Deficits in social cognition, processing speed and executive functioning are often the first and most

significant cognitive symptoms associated with HD, with memory and language functions being impaired later in the disease course.^{227,228} The most significant differences between the HD and ALSci/FTD extramotor phenotypes are prominent psychomotor slowing, smell identification and motor timing deficits, typically present only in HD, caused by primary striatal degeneration.³⁴⁴

Neuroimaging studies have revealed thalamic and fronto-striatal atrophy associated with cognitive presentations in both groups.^{178,407} Patterns of widespread cortical pathology in frontal, parietal and temporal regions correlate with varying cognitive presentations of ALS and HD.^{113,176,253,257,322} Symptoms often present in the premanifest phase of HD, with increasing recognition of a ‘presymptomatic’ phase in *C9orf72*-related ALS-FTSD, where symptoms emerge.⁴¹³⁻⁴¹⁵ Extramotor function in HD appears to deteriorate mostly linearly throughout the prodromal stages of HD, beginning years before clinical-motor onset.^{228,235,378} There has not been clear demonstration of longitudinal progression in symptomatic or presymptomatic ALS, discussed in the previous chapter.

Patients with cognitive/behavioural impairment, particularly apathy in HD^{384,387} and executive dysfunction in ALS^{170,416}, have a more rapid disease course and worse outcomes.^{235,313,417-419} Although distinct extramotor phenotypes have not been definitively characterised, divergent progression phenotypes have been demonstrated based on cognitive function.^{389,390} Alongside the implications for clinical severity and trajectory, early subtle extramotor symptoms can significantly impact psychosocial and occupational functioning. Those affected experience more challenges accessing and engaging with healthcare, as well as managing family, personal and professional relationships. Apathy is a significant and often early behavioural feature of both disorders.^{152,158}

2.2.3 Apathy

A unifying feature of most neurodegenerative disorders is the presence of apathy. Neurobiological, socio-environmental, psychological and individual factors each contribute to the presentation and severity of avolitional symptoms.⁴²⁰ It is also common as part of primary psychiatric disorders, depression in particular.⁴²¹⁻⁴²³ Apathy is defined as, “an observable behavioral syndrome consisting in a quantitative reduction of voluntary (or goal-directed) behaviors” (Levy and Dubois, 2006, p.916). Depression is a broader syndrome, also with multiple neurobiological and psycho-social aetiologies, and is also often present in HD.^{233,379,424} Both apathy and depression are associated with disruption to fronto-basal ganglia circuitry typically involving critical nodes in the salience network.^{422,425}

2.2.3.1 Apathy: Definitions, diagnostic criteria & measures

First classified by Marin^{426,427}, later revised by Starkstein & Leentjens⁴²⁸ and again by Robert and colleagues in 2018⁴²⁹, the diagnosis of apathy necessitates a) a quantitative reduction in goal-directed behaviour from an individual's baseline level of functioning, persisting for at least four weeks, b) affecting two of three domains of apathy, c) resulting in functional impairment and d) not explained by other factors, including environmental changes or substance use. Robert et al.⁴²⁹, propose that the three domains of apathy are, i) behaviour/cognition, ii) emotion, and iii) social interaction. These map largely onto subtypes of apathy first proposed by Levy and Dubois⁴³⁰ which include cognitive, emotional-affective and auto-activation which correspond to distinct PFC-basal ganglia circuitry. This multidimensional approach to apathy has been increasingly adopted, and its relevance in neurodegenerative disease in particular led to the creation of the 'Dimensional Apathy Framework'. This includes domains of apathy similar to those proposed by Levy and Dubois, including Executive, Emotional and Initiation apathy respectively (**Figure 14**). This framework highlights the shared neural architecture of cognition and behaviour, relating apathetic subtypes to Stuss's³³² model for the executive functions (**Figure 16**).⁴³¹

In disorders affecting the motor system, measures of apathy are inflated by a decreases in voluntary movement.^{432,433} The Dimensional Apathy Scale (DAS) was created to control for motor dysfunction, minimising this effect. The DAS has shown clinical utility and validity for distinguishing subtypes and neurodegenerative disorders, including ALS, Alzheimer's disease, and Parkinson's disease.⁴³¹ This framework, rooted in a neuroanatomical model of executive function, may also prove useful for characterising the profile of apathy in HD.

A series of recently published studies from two different groups sought to disambiguate the mechanistic underpinnings of apathy in HD by examining cognitive and other behavioural characteristics of apathetic PwHD. Atkins reported decreased cognitive, but not physical motivation in n=20 premanifest PwHD.⁴³⁴ Assessing decision-making^{435,436}, cost sensitivity⁴³⁷, and time perception⁴³⁸ in small samples of PwHD, together these studies show that cost hypersensitivity—an increased sensitivity to both the cost of effort and reduced appraisal of time delays—rather than a decreased responsiveness to rewards which underlies apathy in HD.^{434,439,440} This finding distinguishes HD from apathy in other neurological conditions like Parkinson's disease and small vessel disease, where reduced reward incentivisation appears to be the primary drive.⁴³⁹ A recent study by Atkins

in n=101 PwHD reported this profile in manHD using the DAS along with other apathy measures.⁴⁴¹

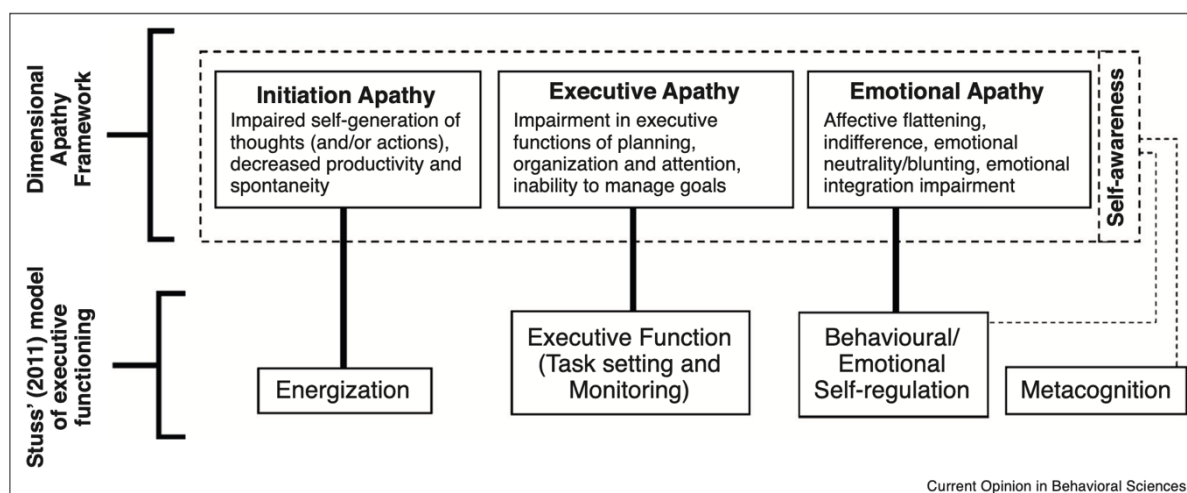


Figure 14: The Dimensional Apathy Framework.

Definitions of the domains and the presentations within each are shown. The neuropsychological model overlap with Stuss's³³² model of the executive functions is also shown. Self-awareness is the fourth component, related to metacognition and orbito-frontal self-regulation calculated by the relative score between self- and informant-rated versions on the DAS. Schematic from Radakovic et al.(2018, p. 45).⁴³¹

2.2.3.2 Neuroimaging apathy

Apathy is associated in particular with medial frontal regions, specifically the ACC. A large resting state-fMRI study by Onoda et al.⁴⁴² found that depression was also associated with functional changes to the ACC, but that these functional changes diverge. They reported a decrease in ACC salience-related processing in apathy, with increases related to excessive detection and integration of information with a negative bias evident in depression.⁴⁴² However, both are multifactorial syndromes and psychological adjustment and contextual factors also modulate expression, complicating differentiation from primary neuropathological processes and construct definitions.

2.3 Shared genetic factors: ALS & HD

Both HD and C9orf72-ALS are repeat expansion disorders. This diverse group of mostly neurological disorders collectively affects ~1 in 3,000 individuals worldwide.⁷⁶ C9orf72 has a higher carrier frequency than *HTT*, but the complex and pleiotropic nature of C9orf72-HRE, discussed above (1.1.6.1.1), is less deterministic than an expanded *HTT* gene. C9orf72 is the commonest cause of HD-phenocopies (Figure 6b).⁴⁴³⁻⁴⁴⁷ A phenocopy

is a disorder which mimics the presentation of a hereditary disease without a causative genetic mutation.^{444,445} A HD-phenocopy is when a patient presents with symptoms of HD but who test negative for *HTT* in the pathogenic range. Further demonstrating an aetiological relationship, the inverse has also been described, where a cases with genetically confirmed HD (*HTT*+) present as clinically diagnosed ALS/FTD⁴⁴⁸ and definite ALS by El Escorial criteria.⁴⁴⁹ The former, a Whole Genome Sequencing (WGS) study, described patients diagnosed with ALS/FTD who had no typical HD symptoms (chorea/dystonia), despite genetically confirmed pathogenic *HTT* expansions.⁴⁴⁸ The authors also validated this with autopsy findings which showed a neuropathological overlap between the disorders with TDP-43-positive, huntingtin-positive and ubiquitin-positive inclusions in fronto-temporal regions, with absence of striatal pathology. They suggest that *mHTT* expansion length may be a phenotypic moderator of ALS.⁴⁴⁸ These cases demonstrate a phenotypic overlap with HD (chorea, cognitive and psychiatric symptoms), and with *C9orf72*-ALS/FTD (rigidity, parkinsonism, apathy, dysexecutive syndromes).⁴⁴⁷

2.3.1 Repeat-expansion disorders & somatic expansion

HD and *C9orf72*-ALS/FTD both exhibit somatic mosaicism and somatic expansion^{82,450}, the focus on which has increased in recent years as the primary pathogenic process, especially in HD.^{287,451} Modifier genes of the *HTT* expansion are being explored as pharmacological targets.

2.4 Why study kindreds in disorders of the motor system?

Studying unaffected relatives allows examination of gene-dose effects, inheritance patterns, and penetrance supporting a better understanding of disease risk and biomarker discovery, that may inform stratification strategies for early therapeutic intervention.⁴⁵² The examination of traits in unaffected relatives supports the identification of endophenotypes as markers of disease susceptibility, which in turn can support studies to uncover unknown genetic mechanisms.¹⁹⁶ Families are a source of often willing participants who may harbour genes of interest associated with conditions in their family history. Gene-negative or familial non-carriers are frequently employed as control participants in presymptomatic studies and increase statistical power. This is a valid and commonly used strategy when the primary aim is to isolate the clinical impact of a specific mutation (i.e.: *HTT* or *C9orf72*), as it helps to minimise the influence of shared genetic or environmental

influences within families. Whilst this is to the benefit of studies in HD, and certain studies in “presymptomatic” ALS, it precludes the potential for kindred studies to elucidate other genetic mechanisms in ALS, as outlined in the introduction with regard endophenotypes.

2.5 The presymptomatic phase & predictive genetic testing

The ability to test for causative gene variants in both disorders affords a unique opportunity to study individuals over time, including the prodromal or premanifest stages, enabling the investigation of disease pathogenesis, the development and progression of cognitive impairment, and the heterogeneity of phenotypic expression around disease onset.⁴⁵³ Approximately 15% of individuals at risk for HD choose to undergo presymptomatic diagnostic genetic testing.^{241,454} Positive genetic testing for *C9orf72* in unaffected individuals does not necessarily offer definitive diagnostic or prognostic information due to reduced penetrance and gene pleiotropy. It may instead be considered as a high-level susceptibility gene, similar to risk classifications in oncology.^{455,456} However, the same principles are applicable, and genetic testing creates an asymptomatic cohort to study the evolution of symptoms.

Testing for the *C9orf72* repeat-expansion in affected ALS patients is commonplace in clinical practice¹¹¹, but testing in at risk-individuals is only available in some countries, and not in Ireland, mostly due to the uncertainty around carriership.^{111,415} Cairns et al.¹¹¹ provide criteria for genetic counselling of patients and their at-risk relatives for known genetic variants in ALS and FTD (*C9orf72*, *SOD1*, *TARDP*, *MAPT*, *GRN*). Service development in Ireland would benefit from insights from these and HD clinical genetics services, which share psychosocial implications.^{457,458}

2.5.1 Why study the presymptomatic phase?

In addition to kindreds, asymptomatic gene carriers are another level at which to study an ‘enriched at-risk’ group, that may uncover biomarkers of disease progression and phenoconversion.²⁷⁹ Phenoconversion refers to the phase transition from unaffected presymptomatic gene carrier, to symptomatic manifest disease. This period is a critical window into understanding disease onset and progression, an ideal time for biomarker development for prognostication and the evaluation of novel therapeutics.^{279,459} Intervention here could delay pathological mechanisms and disability, with the ultimate goal of developing preventative treatments prior to significant neuronal loss (**Figure 15**).^{316,453}

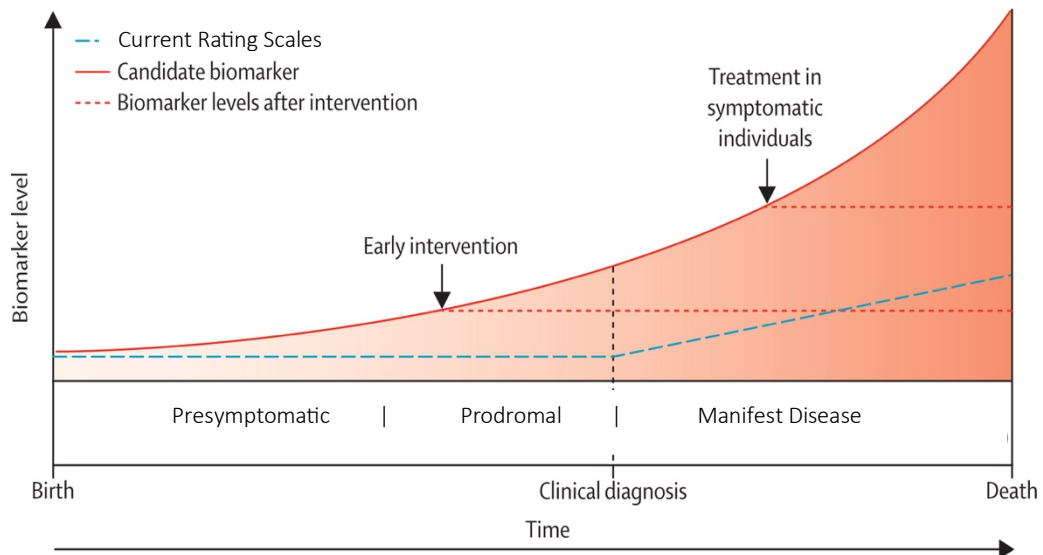


Figure 15: Tracking the lifespan of disease.

Shows a long presymptomatic phase with gradual increase in biomarker levels/pathology and the relationship to current clinical rating scales (e.g.: in ALS and HD). Image adapted from Weir, Sturrock and Levitt.²⁷⁹

NOTE: Presymptomatic intervention that arrests pathological progression prior to clinical diagnosis is an aspirational goal. Also shown is the relative insensitivity of current clinical rating scales, especially to subtle changes prior to clinical onset.

2.5.2 Prediagnostic cognitive/behavioural symptoms

As discussed, cognitive and neuropsychiatric changes can precede motor symptoms by several years in HD and C9orf72-ALS/FTD.^{65,98,157} Identifying early changes and ascertainment of other such biomarkers has implications for staging and diagnosis, risk stratification and prognostication, opening the therapeutic window to the presymptomatic phase (**Figure 15**).²⁷⁹ By characterising this phase more clearly, we improve the precision of natural history models, which underpin clinical trial design, and allow for more proactive models of care.^{223,320,321,460-463} Monitoring of biomarkers, and routine assessments for motor, cognitive, behavioural function are key in research and clinical trials, and encouraged clinically in at-risk individuals.⁴¹⁵

2.5.3 Prodromal staging criteria

Recent efforts have been made to apply staging criteria to genetic disorders with premanifest phases including HD²⁵⁶, FTD^{464,465}, Parkinson's disease⁴⁶⁶ and Alzheimer's disease.³⁰⁶ Staging of presymptomatic and early symptomatic disease supports sample stratification in studies aiming to uncover mechanisms related to onset, and for sample enrichment and evaluation in clinical trials.⁴¹⁵ The HD-ISS provides this framework for HD.

Stage 0 is defined by a positive genetic test^{256,305} with subsequent stages defined by emergence of clinical, neuroimaging and fluid biomarker landmarks (**Figure 11**). Similarly, blood Neurofilament-light (NfL) and atrophy of the ACC are proposed as biomarkers in FTD, with the combination showing excellent diagnostic sensitivity and specificity.⁴⁶⁴ FTD and HD have longer disease courses including relatively long prodromal phases. Such criteria may not provide similar utility in the much shorter transitory phase of ALS. Given its potential however, terminology around the clinically silent phase of *C9orf72*-associated conditions (ALS-FTSD) are discussed widely^{415,464,467}, and frameworks for mild-cognitive/behavioural impairment in presymptomatic ALS have been proposed.^{414,468}

2.5.3.1 Premanifest HD

In HD, the premanifest phase can span decades, depending on when genetic testing is performed.³⁰⁵ Premanifest refers to the whole period, up until clinical diagnosis. It can also be subdivided based on clinical assessment, into “a/presymptomatic” and “prodromal” phases.²³⁷ The prodromal phase is characterised by subtle presentation of possible HD-related changes, but which remain subclinical. Reilmann et al.’s (2014)⁴⁶² Task Force paper recommends this subdivision be based purely on motor symptomatology: presymptomatic stage (no motor signs) and a prodromal stage (detectable motor signs not yet sufficient for diagnosis). However, this overlooks the often present extramotor symptoms.^{298,384,469,470} With further recognition of non-motor symptoms as among the earliest and functionally impairing, Ross et al.²³⁷ proposed adding non-motor signs to the current diagnostic criteria, which would extend into the pre-motor symptom onset phase of HD.³⁰⁸

2.5.3.2 Asymptomatic *C9orf72*-carriership

Familial FTD and ALS studies have used mean age at onset for families to estimate proximity to clinical onset for unaffected gene-carriers.^{65,215,471} This is a crude measure adapted from familial Alzheimer’s disease⁴⁷², but has proven valuable for mapping disease progression in genetically at-risk individuals in lieu of alternative staging biomarkers. It is less reliable in *C9orf72*-carriers.⁴⁷³ *C9orf72* repeat length does not exert the same phenotypic effects that allows prediction models in HD using *HTT* CAG repeat length.⁸²⁻⁸⁴ Given the dimensionality and variability of cognitive and behavioural symptom presentation in relation to ALS diagnosis, which is motor-based, and the marked inter- and intra-familial variability in AoO in ALS, its applicability to ALS is limited.^{6,474} There is a need to develop a refined and disease-specific “time to expected onset” model for *C9orf72*-ALS to support research in this area.⁴¹³

2.5.4 Biomarkers of presymptomatic neurodegenerative disease

Longitudinal studies in the presymptomatic phases of both HD and ALS are needed to understand how and when asymptomatic carriers develop symptoms, and to improve predictive biomarkers. In HD, although mHTT levels are theoretically direct markers of pathology in HD, reduction would reflect target-engagement in the context of huntingtin-lowering therapies, but may not necessarily indicate disease modification.⁴⁷⁵ NfL is a non-specific but sensitive marker of neuronal dysfunction that can be detected reliably in cerebrospinal fluid (CSF), and in peripheral tissues, like blood.⁴⁷⁶⁻⁴⁷⁹ Researchers in both HD and ALS are evaluating the potential of NfL levels as surrogate endpoints in clinical trials and to track presymptomatic and symptomatic disease progression in natural history studies.^{223,255,415,459,480-484} NfL is only detectable immediately preceding symptom onset, evidencing already significant neuronal damage, and so other biomarkers are required for presymptomatic disease stages.⁴⁸⁵ Blood NfL improves prognostic prediction models, useful in clinical trials.⁴⁸⁶ Neuroimaging biomarkers also hold promise, and are discussed further in a subsequent section.

If a biomarker of progression is successfully identified, rapid progressors and those at highest risk of proximal disease onset can be strategically selected to enrich samples, or to “stack the deck” in clinical trials, speeding up the process of detecting treatment effects and therefore drug evaluation.⁴⁸⁷ This practice is known as prognostic enrichment. Stratification of participants to create more homogeneous samples is important in clinical trial design, to minimise interindividual variability. In HD, this is usually achieved by controlling for CAG repeat length and age³²⁰, and more recently with additional HD-ISS staging criteria.²⁵⁶

2.6 Studies in unaffected gene-carriers

There are a number of consortia and large multi-centre studies of the clinically silent phase across C9-ALS/FTD and HD. Studies of the presymptomatic phase in HD are long established, with most biomarker studies and natural history studies following both premanifest and manifest PwHD due to the diagnostic certainty and availability of predictive genetic testing.^{250,263,488} More recently, the same focus has turned to *C9orf72*-positive gene carriers.^{186,215,489} Given the early occurrence of cognitive and behavioural symptoms in HD, and the association to *C9orf72* in ALS/FTD, these clinical symptoms and traits are employed as biomarkers of disease onset, progression and prognosis in the presymptomatic phase. These studies are discussed in subsequent sections.

2.6.1 Studies in premanifest PwHD

Premanifest PwHD present with various subclinical extramotor symptoms which can progress alongside and even precede motor dysfunction. Large multi-centre international cohort studies such as PREDICT-HD⁴⁹⁰ and TRACK-HD^{261,262} have aimed to characterise the timeline of changes in various domains of cognition and behaviour in HD, alongside neuroimaging and fluid biomarker studies across the disease course from premanifest to manifest disease. These deficits correlate with proximity to clinical diagnosis, suggesting a gradual decline in function over time.²²⁸

2.6.1.1 Premanifest HD: cognition

Tabrizi et al. (2011)²⁶² reported longitudinal change across multiple domains over 12 months in premanifest (n=123) and early PwHD (n=122) in the TRACK-HD study. Subtle cognitive changes were detectable in the premanifest group, particularly in tasks of psychomotor speed and executive function. Structural imaging also revealed early atrophy in striatal regions, particularly the putamen and caudate. These changes were seen up to a decade before predicted onset. In the same study, an analysis at 36-month follow-up identified continued progression in premanifest individuals (n=119), particularly in those nearing predicted onset, and most significant in those who received a diagnosis during the study (n=20). Greater decline was observed in cognition (i.e.: Symbol Digit Modalities Test[SDMT]), motor function (i.e.: speeded tapping), and striatal volumes.²⁵⁵ Baseline putamen and caudate volumes on MRI were the strongest predictors of clinical diagnosis, even after accounting for age and CAG-repeat length. Measures of apathy also predicted early functional decline.

An analysis of the PREDICT-HD dataset including (n=738 premanifest PwHD), stratified preHD based on proximity to expected diagnosis.²²⁸ They report increasingly larger effect sizes with proximity across the cognitive battery. The largest effect in the ‘far’ group was identified for affect recognition (d= -0.26) with large effect sizes evident globally in the ‘near’ group, particularly for psychomotor processing speed and speeded executive tasks, but also with smaller effects for crystallised and fluid intelligence.

Paulsen et al. (2014)³⁹² analysed longitudinal data from the up to five annual visits in the PREDICT-HD study (559 preHD, 233 gene-negative controls). PreHD even at DCL=0, showed progressive cognitive decline, or failure to improve (as controls), on the SDMT, Stroop subtests and the Trail Making Test, while controls remained stable or improved. Smell identification was also affected. They conclude that these ‘executive function’

changes correlated with proximity to estimated motor onset, with some deficits emerging over a decade prior.

The HD-Young Adult Study (HD-YAS)^{491,492} follows a cohort of premanifest PwHD an average of 24 years from expected onset (n=64 at baseline), employing extensive cognitive, neuroimaging and fluid biomarker assessments. They found no differences to controls on any cognitive or psychiatric assessment. In a follow-up analysis⁴⁹¹ focused on cognition, differences of small effect were evident on executive set-shifting task, including increased error rates.

A factor analysis of the PREDICT-HD study indicated that of the six factors identified, motor planning/speed and sensory-perceptual processing were the two most predictive of motor-symptom onset.⁴⁹³ Common to most of these studies is that executive dysfunction is reported without reference to the speeded nature of many of the tasks.^{228,262,494} Interpretation of results must consider whether cognitive slowing or motor impairment confound performance on timed executive tasks.

Turner et al.³⁵⁴ performed the first large study of multi-domain social cognition in HD, with group and individual impairments detected relative to controls, with 80% and 22% of premanifest participants meeting their thresholds for below control median and pronounced impairment on the Reading the Mind in the Eyes (RMET) task respectively. Cut-offs were created from their control sample however. Impairments to memory have also been reported in preHD, particularly of verbal memory recall and recognition discrimination.^{495,496}

2.6.1.2 Premanifest HD: psychiatric symptoms

Suicidality has been described in PwHD since its recognition as a disorder.²³² Since then, studies have demonstrated evolving psychiatric profiles, mostly related to depression, anxiety, irritability, and apathy.^{497,498} Symptoms of depression have shown associations to CAG-repeat length and working memory capacity in a small study of premanifest PwHD (n=15).⁴⁹⁹ Meta-analyses report rates of the commonest psychiatric symptoms in up to 80% of those in the premanifest stage.

Epping et al.³⁷⁸ performed a large study (n>1,200 premanifest PwHD and companions) investigating longitudinal psychiatric symptom trajectories using the SCL-90-R. They showed variation with proximity to onset, but symptoms generally increased with 5-year onset probability. Most symptoms showed a sharp increase in the group nearest expected onset.

Hendel et al.⁵⁰⁰ recently reported increased apathy in manifest but not premanifest PwHD (n=40). There was a distinction between cognitive and apathy scores, unexplained by models accounting for depression and motor scores, suggesting it is an independent symptom in HD.

2.6.1.3 Premanifest HD: neuroimaging & biomarker studies

TRACK-HD, PREDICT-HD and the HD-YAS study all describe early biological signs of neurodegeneration in premanifest PwHD. Atrophy is first evident in the putamen up to 24 years from expected diagnosis⁴⁹², with decreases to putamen and caudate volume detectable from 10 years prior, decreasing linearly with proximity to diagnosis.^{255,256} The HD-YAS study, also found increased NfL (plasma and CSF) in alongside the reduced putamen volume, which were both present in the absence of cognitive changes. A later cognition-specific analysis found an association between errors on executive set shifting task which corresponded with altered resting-state fronto-striatal connectivity, shifting from ventrolateral PFC–ventral striatum circuits in controls to dorsolateral PFC–caudate connectivity in premanifest PwHD. A number of smaller studies (n=16 premanifest PwHD) show similarly reduced functional connectivity in prefrontal and parietal areas, as well as the putamen⁵⁰¹, with DLPFC function in particular associated with cognitive decline.⁵⁰²

A larger analysis, combining the TRACK-HD (n=72 preHD) and HD-YAS (n=54 preHD) did not find any subcortical white-matter changes in the cohort 25 years from expected onset, but those on average 11 years from expected onset (TRACK-HD preHD) exhibited reduction to white matter density in caudate and thalamic nuclei that did not deteriorate over the two year study period. White matter fibre density in limbic cortico-striatal circuits were significantly related to apathy symptoms.²⁶⁶

A longitudinal sub-study of TRACK-HD, analysing temporal volumetric brain dynamics over 3-7 sessions up to 10 years in premanifest PwHD who transitioned to manHD (n=49) found widespread cortical and striatal atrophy. Even in peri-manifest and early-manHD frontal, parietal and temporal regions demonstrated significant volume reductions.⁵⁰³ Changes were associated with cognitive and motor outcomes.

2.6.2 Studies of cognition in asymptomatic C9orf72-carriers

The majority of presymptomatic studies in ALS/FTD syndromes have come from large consortia investigating familial FTD, most prominently the Genetic Frontotemporal Dementia Initiative (GENFI) and Frontotemporal Dementia Prevention Initiative (FPI). Findings of these studies are particularly relevant to ALS research for the inclusion of

C9orf72 kindreds as one of the causative genotypes for familial FTD. Although these are FTD kindreds, observation can provide insights into pathogenesis and pathways affected in *C9orf72*-mediated disorders, particularly relevant for the development of cognitive and behavioural symptoms in ALS. The Presymptomatic Familial Amyotrophic Lateral Sclerosis (Pre-fALS) and the Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis Study Group (PREV-DEMALS) studies directly include participants at genetic risk from ALS, and ALS and FTD respectively.

2.6.2.1 Asymptomatic *C9orf72* carriers: cognition

A number of studies have profiled neuropsychological changes in asymptomatic and symptomatic carriers of FTD-related mutations, with a focus on *MAPT*, *GRN*, as well as *C9orf72*.^{65,504,505} Across cohorts, executive dysfunction, slower processing speed, and verbal fluency deficits were common among mutation carriers compared to familial non-carriers. However, differences were typically not specific to genotype with some studies finding associations that others do not.^{65,489,504-506}

One of the first studies from the GENFI study⁶⁵ included 40 symptomatic and 78 asymptomatic mutation carriers (*MAPT*, *GRN*, *n*=18 *C9orf72*). They demonstrated a time-dependent pattern of cognitive and behavioural change, worsening as participants approached the mean age of onset within their family. Significant differences between carriers and non-carriers emerged five to ten years before “expected onset”, particularly in domains of executive function, memory, language, and behaviour. Changes to the insula followed by temporal lobe volume reductions were the first structural changes noted on MRI, which emerged alongside cognitive features.⁶⁵ This time-to-onset approach was also applied to a smaller sample by Benussi et al.⁵⁰⁷ in a study of 52 asymptomatic mutation carriers. Cognitive and behavioural differences were also observed approximately five years before expected onset, supporting Rohrer et al.’s⁶⁵ findings. However, the sample included only *n*=4 *C9orf72*-carriers.

Rohrer et al. (2015)⁶⁵ also performed a sub-group analysis of the *n*=18 asymptomatic *C9orf72*-carriers in their study. Behavioural alterations were the earliest detectable change, emerging up to 20 years before expected onset. By 15 years pre-onset, performance differences were noted on verbal fluency, speed of processing, executive function naming, and story memory. General measures of attention and IQ showed differences from 10 years before expected onset. These findings suggest a prolonged prodromal phase in *C9orf72*-related disease.

The PREV-DEMALS study²¹⁵ compared 41 asymptomatic *C9orf72* carriers with 39 age- and sex-matched familial non-carriers (C9-). Despite a relatively young mean age of 40 (with an average of 25 years to expected onset), mutation carriers demonstrated significantly lower scores in praxis and memory recall.²¹⁵ These findings suggest subtle neuropsychological changes may be detectable decades before clinical onset.

Impaired verbal fluency has been discussed as a characteristic cognitive symptom of ALS. A number of presymptomatic studies have found reduced performance in unaffected carriers of *C9orf72* on letter, or phonemic fluency in particular.^{489,508} Both studies found small effects, with performance remaining in the normal range. Papma et al.⁵⁰⁸ also reported lower scores on Stroop interference tasks in their small cohort (n=18 C9-carriers) compared to family controls. The n=21 asymptomatic C9-carriers in Lulé et al.'s study⁴⁸⁹ had lower fluency and visual memory compared to healthy controls, but not compared to family controls (C9-) and scores were stable over a 12 month period.

Contrary to this, a small study by Lee et al. (2017) found that asymptomatic *C9orf72*-carriers (n=15) and non-carrier family members displayed unaffected cognitive performance. Both groups were found to have similarly high psychiatric symptom history but C9+ carriers had significantly more grey matter volume reduction and reduced white matter integrity compared to controls.¹⁸⁶ These and other studies conclude that *C9orf72* may have neurodevelopmental effects may be evident throughout lifespan, both on imaging studies^{81,186} and retrospective studies of personality characteristics.⁹⁸ Volume reductions in the thalamus are exclusively linked to *C9orf72* in pwALS/FTD^{99,179,407}, also evident to a lesser degree in asymptomatic carriers.^{81,509,510}

Cognitive flexibility, measured by the Trail-Making Test B, were found to differ between asymptomatic mutation carriers (GENFI study), but not the digit span or digit symbol modalities tests.⁵¹¹ Decline across the cognitive assessments (predominantly executive) was related to relative activation of the salience network, predicting future reduction in performance.⁵¹¹ This study did not differentiate different genotype between carriers (*C9orf72*, *GRN*, *MAPT*).

Although social cognitive dysfunction is recognised in pwALS, there are no studies assessing this domain in unaffected at-risk samples.⁴⁶⁷

2.6.2.2 Asymptomatic *C9orf72*-carriers: personality & psychiatric characteristics

A larger GENFI study⁴⁷¹ investigated genotype-specific effects on neuropsychiatric symptoms among 167 symptomatic and pre-symptomatic carriers (n=60 unaffected *C9orf72*-carriers). No significant differences in frequency or severity of depression,

anxiety, hallucinations, or delusions were found between genotypes. Among pre-symptomatic individuals, 15% reported depression and 12% anxiety, with hallucinations or delusions reported by only one *C9orf72* carrier (3%). There was no control group in this study however, limiting interpretation.

A large retrospective qualitative study of a cohort of bvFTD patients found lifelong personality traits more common in *C9orf72*-positive cases, preceding disease onset.⁹⁸ Lower empathy and higher levels of compulsive personality traits characterised this presymptomatic profile. A similar but prospective study of psychiatric traits in unaffected C9-carriers found much higher rates of depression, post-traumatic stress disorder and substance abuse.⁵¹² This study also replicated findings of the retrospective qualitative study by Gossink et al.⁹⁸ by demonstrating reduced empathy prospectively in their sample (n=21 unaffected C9+). Empathy was associated with lower MMSE scores but all were above the clinical cut-off.⁵¹² Case study reports have described initial presentations of *C9orf72*-bvFTD infrequently being characterised by profound psychotic disorders, misdiagnosed as schizophrenia, indicating the relative commonality of psychiatric disturbance in the *C9orf72* phenotype.¹³⁻¹⁵

A later examination of 221 symptomatic and asymptomatic FTD-gene-carriers (C9: n=138) within the GENFI study found high rates in affective (depression, anxiety, sleep disturbance) symptoms in carriers, up to 40 years before expected onset of FTD.⁹⁹ Psychotic symptoms were commonest in *C9orf72* carriers, with very few in other genotypes, and were linked to right thalamic atrophy, suggesting a specific neuroanatomical signature.

2.6.2.3 Asymptomatic *C9orf72*-carriers: neuroimaging studies

GENFI's first published study also included neuroimaging data on 78 asymptomatic mutation carriers, 18 of whom were *C9orf72* positive.⁶⁵ Compared to 102 non-carriers from the same families, the first observable structural differences were detected in the insula and temporal lobe, up to a decade before estimated symptom onset. At five years prior, volume differences were evident in the frontal lobe, subcortical regions, and whole brain. Changes had also emerged in the parietal lobe and cingulate cortex at the time of expected onset.⁶⁵ Other studies mirror these findings, with structural abnormalities detected across frontal, orbitofrontal, insula, temporal, anterior cingulate, thalamic, parietal and cerebellar regions in presymptomatic gene carriers.^{467,485,513,514} Unique neuropathological features are evident by genotype. Thalamic and cerebellar atrophy are the most distinct in presymptomatic C9-carriers.^{509,513} Insula and temporal lobe atrophy

are also prominent in *C9orf72* carriers, distinct from GRN carriers in relation to progression of apathy symptoms.⁵¹⁵

White matter integrity is significantly disrupted in *C9orf72*-carriers (n=38) compared to non-carriers in the PREV-DEMALS study, with abnormalities found in multiple frontotemporal pathways and corticospinal tracts⁵¹⁶ Projections to the inferior frontal and orbitofrontal cortices are also altered in unaffected *C9orf72*-carriers compared to healthy controls, indicated by reduced fractional anisotropy, which correlated with verbal fluency performance.⁴⁸⁹

Using 18-labeled fluorodeoxyglucose ([18]FDG) positron emission tomography (PET), asymptomatic *C9orf72*-carriers from ALS kindreds showed clusters of relative hypometabolism in frontotemporal, and thalamic regions compared to healthy controls.⁴⁸⁵ This sample was likely close to onset as over half demonstrated UMN signs, and nearly one third had detectable cognitive impairment. Regional hypometabolism was evident for in 82% of participants, including those who did not show elevated CSF NfL or detectable executive deficits.⁴⁸⁵

A large imaging study⁵¹⁰ which included 54 symptomatic and 83 asymptomatic carriers of *C9orf72* from FTD kindreds (GENFI study) showed a more rapid but linear reduction in brain-wide cortical thickness throughout adulthood for carriers compared to controls. Thinning corresponded most highly to regions of pathology in symptomatic patients, originating in medial frontal and temporal regions. Changes were evident from as early as 30 years old, particularly in ventrofrontal regions.⁵¹⁰ Presymptomatic carriers did not differ from controls on cognitive or behavioural screening however, and there was no association with time to expected onset.

A large longitudinal study followed at-risk relatives from *C9orf72*-positive ALS kindreds for up to 13 years.⁸¹ Five (of 58) unaffected C9+ carriers in their sample subsequently developed ALS during the study period. They found regional specific longitudinal changes to key cortical (frontal, temporal, parietal, cingulate) and sub-cortical structures (thalamus, hippocampus, amygdala) up to six years prior to development of ALS.⁸¹ Individualised atrophy signatures of cortical thinning identified disease susceptibility, underscoring the potential for MRI to complement or even precede biofluid markers (e.g.: NfL) in detecting prodromal ALSFTSD changes, offering a longer diagnostic and intervention window.

In a small structural imaging study in a cohort of at-risk unaffected ALS relatives, Bede et al.⁵⁰⁹ found subcortical changes most characterised unaffected *C9orf72*-carriers most evidently in the thalamus, but also the hippocampus. There were no cortical grey

matter alterations evident in *C9*-carriers. Segments of the thalamus most affected in this study are associated with limbic and executive circuits.⁵⁰⁹ These findings mirrored those in the GENFI study.⁶⁵ Asymptomatic *C9*-carriers from familial-FTD kindreds showed thalamic, insula and posterior cortical changes up to 25 years before estimated onset, with cerebellar involvement coming later in the presymptomatic phase.⁶⁵

A large imaging study within GENFI used resting state fMRI analysis to capture changes in functional network dynamics as indicator of phenoconversion in genetic-FTD (n=119 unaffected *C9*-carriers).⁵¹¹ They reported that a relative increase to salience network activation predicted both neuropsychological test scores and phenoconversion in familial-FTD. Altered dynamics were evident prior to onset, late in the presymptomatic phase, and so the authors proposed that these patterns are indicative of an immediate biomarker of phenoconversion.⁵¹¹

Many suggest that *C9orf72* has neurodevelopmental effects, and this is reflected in presymptomatic imaging studies where unaffected carriers have significant changes to brain morphology relative to controls, and asymptomatic carriers of other genotypes that do not appear to be time-dependent.^{467,509,517} Some studies however did not find morphologic difference in unaffected *C9*-carriers.^{467,518}

2.7 Clinical trials

Disease modifying therapies have yet to be developed for ALS and HD, with the exceptions of Riluzole and Tofersen, both for ALS. In disorders with monogenic causes, precision medicine is revolutionary in terms of pharmacological intervention. Tofersen for *SOD1*-ALS and Spinraza for Spinal Muscular Atrophy (SMA) exemplifies successful gene-to-therapy translation.⁵¹⁹⁻⁵²¹ Despite the 32 years that the causative HTT mutation has been known, and substantial developments in medical technologies and therapeutics, there is still no disease modifying treatment on the market. Gene-silencing therapies neutralising aberrant RNA or reducing the effects of misfolded protein have been ineffective in both ALS and HD to date, despite showing target engagement.^{106,475,522} Failure of trials in *C9orf72*-ALS and HD thus far are most likely due to an incomplete understanding of pathomechanisms. Target engagement and the pharmacodynamic success of therapies in *C9orf72*-ALS¹⁰⁶ and HD^{475,522} demonstrate the capability of drug-developers and the disease-modifying potential of similar therapies. Should the findings of Handsaker and colleagues²⁷¹ be replicated, it suggests that DNA-based therapies and modulators of neuronal somatic expansion should be the focus of therapeutic development in HD. This

work may be widely applicable in other repeat expansion disorders, including *C9orf72*-ALS.

2.7.1.1 Presymptomatic clinical trials

Benatar et al.⁶² emphasise successes like Tofersen indicate the viability of biomarker-informed presymptomatic treatment trials for *SOD1*-ALS, potentially opening the door to other diseases too.⁴⁸⁰ The recently published series on common genetic forms of ALS together crystallise a shift in ALS research from descriptive neuropathology to mechanistic understanding and targeted intervention, particularly within the context of TDP-43 proteinopathy, genotype-specific mechanisms, and early intervention.^{51,61-63} Early intervention may arrest the onset of disease, as it does in SMA with Spinraza. In theory, treatment in the presymptomatic phase of genetic disorders maximises disease- and disability-free life, reducing the number of Disability-adjusted life years (DALYs), with the potential to silence the disorder entirely, before manifestation. However, efficacy of drugs in asymptomatic *C9orf72* and *HTT* carriers is unproven and difficult to demonstrate.⁴¹⁵ Identification of reliable and specific biomarkers for ALS in early and premanifest phases is therefore imperative. They will enable early identification and tracking of patients in clinical trials, and evaluation of treatment efficacy at the earliest stages where intervention to arrest disease processes has the highest potential. Due to the prominent extramotor involvement, research and clinical trials in *C9orf72*-ALS/FTD and HD should incorporate standardised and reliable motor and cognitive-behavioural surrogate outcome measures.

2.8 How neuropsychological theory informs this thesis

2.8.1 Differential deficits & domain specificity in neurodegenerative disease

Miyake et al.⁵²³ posit that three “latent constructs” contribute in different proportions to the success of a complex executive task—shifting, updating and inhibition. Many widely used tests of executive function assess multiple ‘latent constructs’ within executive functioning, and therefore, disambiguating the particular processes that are impaired in cohorts of HD and ALS patients have not been clearly defined. Furthermore, the neural correlates of the executive functions are also poorly understood due to the “task impurity problem”^{523,524} which can confound neuroimaging findings due to the supervisory role of many cognitive functions that the executive functions have responsibility for, and

which can be seen by the extensive network of reciprocal connections from the prefrontal cortices to almost all regions of the cortex and subcortical structures.^{332,523}

Foundational to clinical neuropsychological assessment, the importance for determining differential deficits in neurodegenerative disease is highlighted by Crawford.^{525,526} Differential deficits refer to disproportionate impairments in specific cognitive domains relative to general intellectual decline. Their identification is crucial for delineating disorder-specific cognitive profiles, informing differential diagnosis, and detecting early, selective impairments.

2.8.2 Executive functions

In cognitive tasks requiring the maintenance of goal-based behaviour, monitoring for errors and control of inhibitory action, the dorsolateral prefrontal cortex is differentially activated, and a key region in both HD and ALS with cognitive impairment. Attentional and response production ability are governed by the ACC and superior medial circuit underlie apathy, also relevant for the symptoms of volitional behaviour and apathy.^{142,332,527-529}

2.8.3 Stuss' model of executive functions

Due to its neuroanatomical basis and its direct relevance to fronto-striatal networks, central to the neuropsychological profiles of both ALS and HD, Stuss' model of attentional and executive functions is employed as a conceptual framework for interpreting and contextualising executive dysfunction in these studies (**Figure 16**).^{332,333} Where primary degeneration in the striatum likely interferes in all fronto-striatal pathways within Stuss' model in HD, degeneration of the superior medial circuit underlying Stuss' 'Energization' construct are the earliest and most significantly impacted in both ALS and HD, and which later impairs the 'Executive Function' component of task setting and monitoring. Disruption to the superior medial circuit is associated with decreased reaction times and poorer performance on tasks requiring cognitive speed, especially relevant in global processing speed impairments evident in Huntington's disease. As such, timed tasks may be particularly sensitive in the neuropsychological assessment of HD.²³⁰ Motor dysfunction must be taken into account when doing so however, which would confound cognitive scores. Interference in the superior medial circuit is also thought to be associated with impaired verbal fluency, and particularly word generation ability. Both phonemic and semantic fluency are impaired in HD, and phonemic switching—the ability to shift between phonemic categories—may underlie performance in both tasks.¹⁴⁰ So too

can dysfunction to the superior medial circuit underlie apathy, with the anterior cingulate areas of this network being the most significant.^{142,529}

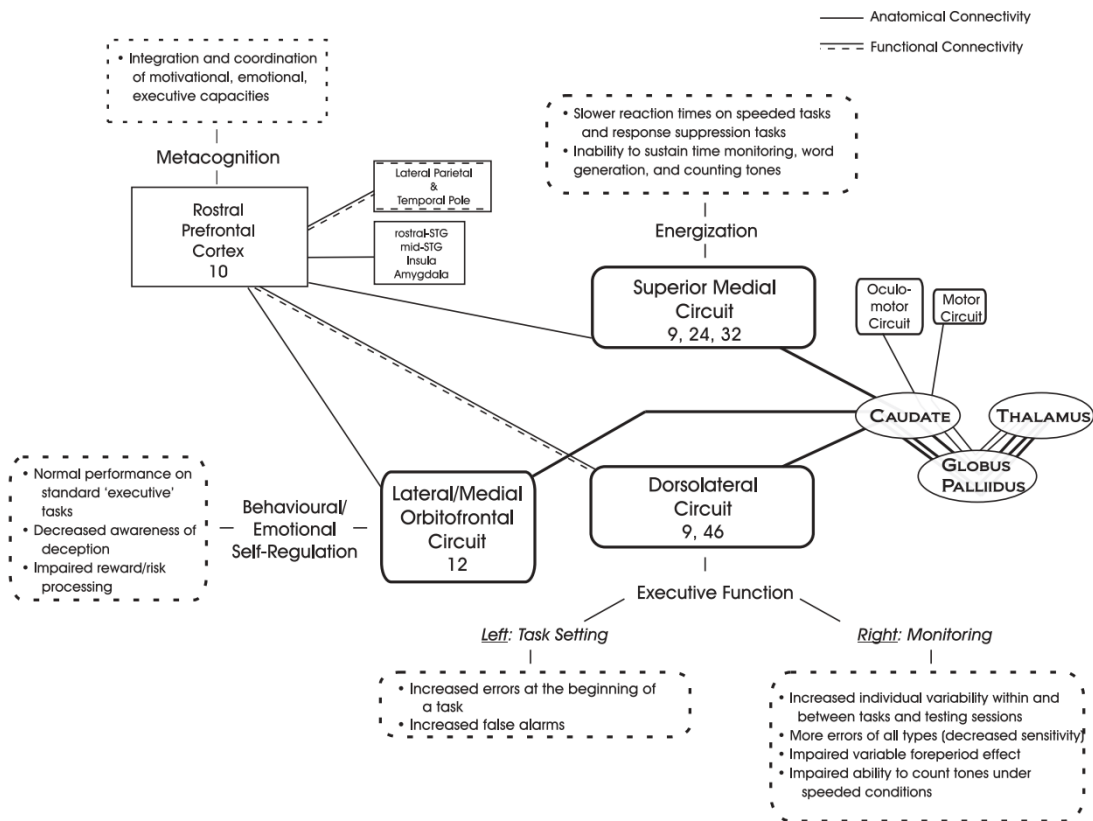


Figure 16: Stuss' model of the executive functions.

Schematic of Stuss' functional-anatomical model of frontal-subcortical networks underlying executive functioning. From Stuss (2011).³³²

2.9 Conclusions

The first two chapters provide comprehensive overview of the clinical, genetic and neuropsychological features of ALS and HD. ALS is emphasised as a multisystem disorder which overlaps clinically, pathologically and genetically with FTD. The first section highlights the presence of cognitive and behavioural symptoms in up to 50% of pwALS, and the influence of *C9orf72* on phenotype and prognosis. Phonemic verbal fluency performance, as a sensitive marker of ALSci, is an important candidate for indexing disease liability as a putative cognitive endophenotypes in unaffected fALS relatives.

The next section introduces HD, as a genetically determined, multisystem disorder with a long prodromal phase marked by executive dysfunction, apathy, and social cognitive impairment. These cognitive/behavioural symptoms often precede motor signs and better predict functional decline. Despite the clear genetic cause, HD shows substantial clinical heterogeneity, with growing evidence for distinct cognitive-behavioural subtypes. Cognitive changes can emerge up to 15 years before motor onset, indicating a prolonged prodromal phase that is measurable and clinically relevant.^{228,336} Similarly, neurodevelopmental and presymptomatic cognitive-behavioural changes are increasingly recognised in *C9orf72* expansion carriers.

Similarities between HD and *C9orf72*-ALS have been presented with the potential for both disorders to mutually benefit from shared methodologies and lessons within a clinical neuropsychology, clinical genetics, clinical trials and research contexts discussed. Clinical trials for genetic therapies, mostly ASOs, are ongoing, aiming to arrest disease progression by silencing aberrant gene function. These trials require robust measures of cognitive and behavioural symptoms common in the disease phenotypes, which can function as clinical cognitive biomarkers. Research is therefore shifting focus earlier in the disease course with an increasing recognition of the importance of characterising the ‘presymptomatic’ phases through the study of clinical evolution of symptoms from asymptomatic gene-carrier to manifest disease. Together studies highlight a measurable and prolonged prodromal phase in HD. Insight into this critical period for disease manifestation with longitudinal studies of asymptomatic gene carriers are crucial, and may help define critical windows, hopefully, for intervention with disease-modifying therapies. Detailed phenotyping in this phase also supports the standardisation of diagnostic criteria and the development of reliable tools to monitor early cognitive and behavioural change.

Apathy is a common behavioural symptom to both, with new clinical frameworks aiming to elucidate overlapping neuroanatomical cognitive networks. Cognitive impairment^{2,235,313,417} and apathy^{387,418,419} are negative prognostic indicators in HD and ALS, often explaining more of the variation in disease progression than motor features, and causing the most significant impact to patient and caregiver wellbeing.

Together, these reviews reinforce the importance of neuropsychological assessment in neurodegenerative disease, for early detection, phenotyping, and disease monitoring of evolving extramotor profiles in genetic disorders. As research evolves, diagnostic frameworks must adapt to better capture the complexity and prognostic value of non-motor symptomatology in genetic neurodegenerative disorders.

These introductory chapters underscore the value of studying asymptomatic relatives and support a transdiagnostic approach, laying the foundation for subsequent chapters, which examine emerging and early neurocognitive profiles, considerations for neuropsychological assessment, and the role of endophenotyping in identifying heritable, subclinical markers of disease liability. The next chapters build on these insights, presenting a series of studies which integrate findings from ALS and HD to examine shared and distinct mechanisms of extramotor dysfunction. Studies reviewed and information discussed in these introductory chapters has supported the generation of hypotheses which underlie this research. The next chapter will outline the rationale, aims and hypotheses of these studies.

3 Chapter 3:

Aims and Objectives of this Thesis

3.1 Overall thesis objectives

Neurodegenerative diseases of the motor system, such as HD and ALS, are traditionally defined by their respective core motor symptoms. An increasing body of research highlights that non-motor cognitive and behavioural changes often precede or accompany motor onset, suggesting a broader and more complex phenotype than previously recognised. Clinical neuropsychology plays a vital role in characterising these extramotor manifestations and in identifying early markers of disease, with implications for diagnosis, staging, prognosis, and clinical trial readiness.

The research in this thesis builds upon neuropsychological and clinical expertise established by members of this research group for the evaluation of cognition and behaviour in pwALS.^{2,38,68,158,163,170,183,188,530-532} These validated methods are extended to HD and to unaffected relatives of pwALS, with the goal evaluating the utility of a transdiagnostic neuropsychological approach to phenotyping in early neurodegenerative disease stages and endophenotyping for disease liability in at-risk populations. This thesis investigates the cognitive and behavioural manifestations of HD and ALS across disease stages, and the traits of those at risk. It further examines how contextual (e.g.: testing environment) and individual (e.g.: motor or processing speed limitations) factors influence neuropsychological performance, and introduces novel methods designed to enhance the sensitivity of cognitive outcome measures.

A domain-based neuropsychological framework is adopted, aligned with the approaches of Phukan et al.² in pwALS and Julayanont et al.²²⁷ in PwHD. This work is grounded in clinical neuropsychological theory on differential deficits^{525,533}, with Stuss's³³² model of executive functions providing a functional-anatomical methodological framework. Informed by a critical review of the neuropsychological literature, this thesis prioritises domains with established relevance in clinical and research settings where confirmation and clarity is warranted. Specifically, verbal fluency, apathy, and social

cognition as candidate markers of early cognitive dysfunction and disease liability in HD and ALS. The broader aim is to advance a domain-based, dimensional model of cognitive and behavioural change in heterogeneous neurodegenerative disease, with ALS and HD as exemplars.

3.1.1 Potential contributions of this research

Neuropsychological research of this type could impact the field in many ways:

- i) Clinical care: Profiling cognitive and behavioural symptoms can support personalised multidisciplinary care.^{534,535} Early detection aids communication, informed consent, and future planning. Understanding how distress and environmental factors affect test performance may help improve clinic visits and assessment settings.
- ii) Clinical genetics: Longitudinal data from presymptomatic *C9orf72* carriers can improve our understanding of penetrance and risk, informing genetic counselling, risk communication, and presymptomatic testing frameworks. The broader impact of familial ALS, and HD, may support improved care pathways for at-risk relatives.
- iii) Sub-phenotyping heterogeneous diseases: ALS and HD are heterogeneous in their presentation and progression. This research refines cognitive and behavioural subtypes, especially in presymptomatic phases, helping to define meaningful clinical subgroups.
- iv) Diagnosis: the current work will support the creation of clinical extramotor diagnostic criteria for HD^{307,308,462}, as has been done in ALS.¹⁶ It facilitates recognition of the full spectrum of cognitive and behavioural change in HD, and also potentially contribute to classification criteria for presymptomatic *C9orf72*-ALS.
- v) Prognosis: Cognitive and behavioural symptoms are linked to poorer outcomes in ALS and HD. Identifying these features during pre- and peri-manifest stages may support clinical prognostication, sample enrichment and research monitoring.
- vi) Neuropsychological assessment: Motor impairments are known to influence cognitive test validity in ALS; this research highlights the need for equivalent rigour in HD, where psychomotor speed remains the dominant cognitive endpoint.
- vii) Biomarker identification: Neuropsychological profiles may act as cost-effective, accessible markers of disease risk and progression. Longitudinal data can indicate patterns of disease spread beyond the motor system, complementing fluid or imaging biomarkers.

- viii) Clinical trials: These data can also aid in stratifying participants to reduce sample heterogeneity and boost statistical power. Identifying participants more likely to respond to treatment may enhance sensitivity and reduce trial costs. Instruments such as the ECAS, currently used in ALS clinical trials, may be extended to HD trials if validated for construct reliability and domain sensitivity.
- ix) Caregiver and family Support: cognitive and behavioural symptoms are strong predictors of carer strain and reduced wellbeing. Greater awareness and early recognition of cognitive and behavioural changes in heterogeneous heritable disorders of the motor system could inform the development of education and support programmes for families.
- x) Genetics research: discovery of endophenotypes in ALS can improve power in genetic studies, facilitating gene-identification.

3.2 Aims & objectives

This thesis aims to investigate cognitive and behavioural profiles across ALS and HD, including presymptomatic, manifest, and at-risk populations. Eight primary objectives guide the research.

3.2.1 Aim 1: Online Validation

To facilitate study continuation throughout and after the Covid-19 pandemic, Aim 1 sought to validate the online neuropsychological test battery against the standard in-person administration, in a test-retest reliability study of controls.

Hypothesis 1.1: An online version of the extended neuropsychological assessment battery will be a valid alternative to the standardised in-person assessment.

3.2.2 Aim 2: Determine the impact of the clinic environment and recent diagnosis on ECAS performance in pwALS

Aim 2 continues to examine the testing environment, evaluating how cognitive screening outcomes are impacted by clinical environment and recent diagnosis. The impact of distress, behavioural symptoms, and clinic-related factors on cognitive and behavioural screening scores for pwALS undergoing baseline assessment in the National MND Clinic.

Hypothesis 2.1: Self-reported distress, measured by the Distress Thermometer, will be found at high rates in newly diagnosed pwALS;

Hypothesis 2.2: Subjective distress will have multifactorial causes, and will be associated with greater travel distance, longer clinic wait times, closer proximity to diagnosis, and higher levels of self-rated ‘tiredness’;

Hypothesis 2.3: Patients with increased behavioural symptoms (on the BBI) report lower levels of distress, associated with lower insight;

Hypothesis 2.4: Distress will be significantly and inversely related to ECAS performance, when controlling for other factors (age & education).

3.2.3 Aim 3: Identify profiles and influence of apathy across neurodegenerative diseases

Aim 3 of this thesis was to evaluate apathy longitudinally, and investigate the relationship between multidimensional apathy domains and cognitive function across diagnostic categories. Cognitive screening and extended neuropsychological assessment are performed with pwALS in the MND Clinic and PwHD in HD-Cognition study.

Hypothesis 3.1: Apathy profiles (Executive, Emotional, Initiation) will differ between HD and ALS, with PwHD exhibiting more symptoms of Executive apathy;

Hypothesis 3.2: Although the two often overlap, apathy presents independently of depression in HD;

Hypothesis 3.3: Domain-specific apathy scores will be associated with performance on ECAS (pwALS) and composite cognitive scores (pwHD);

Hypothesis 3.4: Apathy and fatigue relate to cognitive function, but do not account fully for reduced performance in PwHD.

3.2.4 Aim 4: Evaluate the presence and stability of cognitive endophenotypes in first- and second-degree relatives of patients with familial ALS.

Aim 4 develops the research around cognitive endophenotypes of familial ALS and longitudinal evaluation of unaffected *C9orf72* gene carriers within the MND Family study.

Hypothesis 4.1: the effect, demonstrating reduced performance on specific cognitive tasks in unaffected fALS relatives, will be replicated. Strongest effects will present for tasks most associated with ALSci;

Hypothesis 4.2: these deficits will show temporal stability in the longitudinal aspect, indicative of a cognitive endophenotype;

Hypothesis 4.3: at the group level, unaffected *C9orf72*-positive fALS relatives will not differ in performance from *C9orf72*-negative relatives;

Hypothesis 4.4: a proportion of unaffected *C9orf72*-positive fALS relatives will show significant decline in cognitive test scores (particularly phonemic verbal fluency), indicative of an early disease cognitive biomarker, rather than a stable endophenotypic trait.

3.2.5 Aim 5: Investigate social cognitive function in PwHD as early marker of disease onset

Aim 5 endeavours to determine the most sensitive markers of cognitive change in pre- and peri-manifest Irish PwHD.

Hypothesis 5.1: Premanifest HD participants will display cognitive profiles intermediate to manifest HD and control participants at the group level, especially in social cognitive function and speed of processing;

Hypothesis 5.2: PwHD will perform worse on new battery of social cognitive tasks (ACS-SP) than controls, with larger effects on tasks with less naturalistic or context-rich information (e.g.: Affect Naming);

Hypothesis 5.3: Social cognitive function, measured by the ACS-SP, will decline a) with increasing estimated CAG-associated disease progression (CAP100), and b) longitudinally over the study period in PwHD, indicative of its sensitivity to disease progression.

3.2.6 Aim 6: Assessment of the factors influencing neuropsychological assessment in neurodegenerative disease

Aim 6 explores the factors across studies which impact cognitive test scores, with a view to improve test reliability and validity.

Hypothesis 6.1: Performance on the RMET will be significantly affected by verbal IQ, particularly below a critical threshold;

Hypothesis 6.2: In addition to **Aim 3**, uni-dimensional and multidimensional apathy is hypothesised to impact cognitive performance in HD and pwALS;

Hypothesis 6.3: Speed and motor function impact neuropsychological test scores in premanifest and early HD: improved performance is evident after appropriate control (contrast scores for executive function and verbal fluency indices).

3.2.7 Aim 7: Create and test a new measure of the differential deficit between phonemic and semantic fluency (RFI).

Aim 7 introduces and tests a novel measure for detecting selective verbal fluency deficits: the Relative Fluency Index (RFI).

Hypothesis 7.1: The RFI will distinguish unaffected fALS relatives from controls by revealing a disproportionate phonemic fluency deficit;

Hypothesis 7.2: The RFI will not distinguish PwHD from controls due to generalised fluency and processing impairments in HD.

3.2.8 Aim 8: Assess the validity, sensitivity and specificity of a cognitive screening measure for use in Huntington's disease

Aim 8 will evaluate the utility of the ECAS for use in the cognitive screening of HD, with emphasis on premanifest HD participants over time.

Hypothesis 8.1: The ECAS will be sensitive to early cognitive changes in PwHD;

Hypothesis 8.2: The ECAS will demonstrate sensitivity to longitudinal decline in PwHD.

4 Chapter 4:

Methodology

4.1 Study design

This thesis comprises a series of studies with minor methodological differences. Overall, an observational, quantitative, prospective, case-controlled design was employed. In some instances, population-based and family-based designs were used, incorporating both cross-sectional and longitudinal elements. This section provides an overview of the general methodology; study-specific details are presented in subsequent chapters.

Across all studies, comparisons were made between patients and/or at-risk family members, including asymptomatic/presymptomatic gene-carriers and healthy control participants. A cross-sectional study of incident, clinic-based ALS cases is presented in **Chapter 5** and **9**.

4.2 Primary studies

The work on which this PhD is based comes mostly from two pre-established studies: the *Pleiotropy / MND Family Study* and the *HD-Cognition Study*. The aims of this PhD project align with the initial study goals, extending them through a focused examination of early cognitive and behavioural markers in prodromal stages, longitudinal trajectories, the validation neuropsychological measures and replication of novel findings. To ensure continuity during the Covid-19 pandemic, remote protocols for neuropsychological assessment were developed and validated, detailed in **Chapter 5**. Presented in this thesis is the product of extensive new data collection and analyses that contribute to the broader interpretation and impact of the findings. Three main datasets were used across the thesis, depending on the research question in each chapter. They are:

1. *Genetic Pleiotropy in ALS Kindreds (Pleiotropy / MND Family Study)*;
2. *HD-Cognition*;
3. *MND Clinic patient database*.

4.3 Study Inclusion / Exclusion Criteria

For all studies, inclusion and exclusion criteria were applied as follows (**Table 5**):

Table 5: *Inclusion / Exclusion criteria for all studies used in this thesis*

Inclusion Criteria	
All studies	✓ Aged 18 or older.
ALS relatives	✓ First- or second-degree relative of incident Irish ALS patients diagnosed January 2015 to December 2017; ✓ First- or second-degree relative of Irish familial ALS patients diagnosed January 2008 to December 2023 (Byrne criteria¹⁰⁸ for possible, probable and definite fALS;
ALS patients	✓ Diagnosis of Possible, Probable or Definite ALS according to El Escorial Criteria; ✓ Resident in the Republic of Ireland for at least three years prior to diagnosis; ✓ Baseline cognitive/behavioural screening assessment in National MND Service between February and October 2023.
HD participants	✓ Clinically and/or genetically confirmed diagnosis of Huntington's disease; ✓ Individuals at risk of HD (have first degree relative) but who are asymptomatic (including Genotype Negative, Premanifest and Genotype Unknown individuals).
Healthy Controls	✓ No family history of MND, FTD or HD.
Exclusion Criteria	
All studies	✗ Active psychiatric disease which inhibits consent or ability to participate in the assessment process; ✗ A history of other neurologic conditions that affect cognition, such as Traumatic Brain Injury or Stroke; ✗ A history of a learning disability or developmental disorder (including dyslexia); ✗ Current use of high-dose psychoactive medication affecting one's ability to engage in neuropsychological and neuropsychiatric testing; ✗ Non-native English speaker; ✗ Illiterate.

4.3.1 Longitudinal study design

Follow-up assessment was performed on test population (PwHD and unaffected fALS relatives), as well as controls. Participants were made aware of the longitudinal design during the consent process (if relevant to the study). They were informed at the end

of each appointment and verbal consent for follow-up by phone and email was obtained. Participants who could not be contacted three times or did not respond to a letter / email requesting contact were deemed “lost to follow up”. Other reasons for discontinuing participation included death, ill-health and participant declining follow-up (request to withdraw from longitudinal study).

Procedures and intervals differ by study, and are outlined in relevant study chapters. Due to variation in interval time, interval time is evaluated before each analysis of interest with a view to statistically control for it if associated with dependent variable. It is not included if no effect is present to preserve statistical power. Intervals in all studies are over 12 months, and therefore effects related to interval time are not expected.^{536,537}

4.4 Participants

All prospective participants since 2020 were recruited by the author (CP), and in some cases, by research assistants and another PhD student working on the MND Family study. Recruitment flow-charts for the MND Family Study and HD-Cognition Study are presented in their respective chapters.

4.4.1 *ALS Kindred Recruitment*

Participants in the MND Family Study were recruited through their patient relatives in the National MND Service at Beaumont Hospital and through the Irish MND Register in line with ethical procedures. fALS is defined under the Byrne criteria.¹⁰⁸ The fALS relative group were first- or second-degree relatives of patients who met these criteria. The sALS relative group comprised relatives of pwALS who did not meet the Byrne criteria for fALS (no discernible family history of ALS). Relatives of all incident cases from 2015 to 2017 and relatives of fALS and *C9orf72*-positive patients from 2012 to 2024 were invited to participate in the study. Phase 1 refers to all participants recruited / data collected in baseline MND Family study up until March 2020. Phase 2 refers to all data collected since then, which includes longitudinal and prospective arms.

4.4.1.1 *fALS Longitudinal Study Recruitment*

For the longitudinal aspect of the MND Family Study, those fALS relatives who took part in the initial phase of the study (2015-2020) were invited to repeat study procedures between March 2021 and November 2024. Many declined follow-up to complete an extended neuropsychological assessment in Phase 1, and therefore only had cognitive

data from the ECAS available. The ECAS-only cohort were also contacted to capture extended neuropsychological data from this group as prospective participants in Phase 2, improving the representation of the population.

4.4.2 HD-Cognition recruitment

Participants in the HD-Cognition study were recruited by convenience sampling through the Enroll-HD Study^{538,539}, which has a study site in Beaumont Hospital, board postings in Beaumont Hospital and the Huntington's Disease Association of Ireland. Baseline assessments for the first 23 participants were carried out between June 2019 and March 2020. The remainder of the assessments (baseline and follow-up) were collected after the creation of online procedures due to the Covid-19 pandemic between March 2021 and November 2024.

4.4.2.1 HD-Diagnostic Criteria

PwHD were categorised by genotype through pre-existing clinical genetics services. Copies of genetics reports were requested for confirmation. Manifest HD participants were those who had received a clinical diagnosis of motor-onset HD from a qualified neurologist (data obtained through chart-review). For the majority who were also Enroll-HD participants, motor symptom data from annual visits were reviewed in October 2024 to confirm diagnostic criteria and time-stamped for the final analyses. Manifest participants are those rated as 4 on the Diagnostic Confidence Level (DCL) scale.²⁸⁸ Premanifest participants were those who carried the pathogenic expansion, but had not received a clinical diagnosis, had no overt motor symptoms and had a DCL ≤ 3 . Gene negative HD family members (those who had received a negative clinical genetic test) were also included as control participants in the HD-Cognition study.

4.4.3 Healthy Control Recruitment

A sample of healthy control participants matched for age, sex and education were contacted through existing case control studies via the Academic Unit of Neurology Control Participant Bank at Trinity College Dublin, and established recruitment networks such as from postings within Beaumont Hospital, volunteer.ie, local advertising and community groups. All healthy controls met exclusion criteria and had an absence of MND, HD and FTD in their family history. In order to maximise numbers in the control groups and due to the considerable overlap in the assessments, several controls additionally completed the non-overlapping tests in a second session to allow inclusion in both HD-

Cognition and the MND Family Study. In both primary studies, non-biological family members were also included as controls if they met the inclusion/exclusion criteria.

4.5 Genetic testing

4.5.1 Genetic testing: pwALS & kindreds

Blood samples were collected from pwALS, relatives of pwALS and control subjects participating in the MND Family Study for DNA extraction and genetic analysis. Each sample was assigned a unique identifier at the point of collection and stored in a freezer at the Trinity Biomedical Sciences Institute. Genetic screening for the pathogenic hexanucleotide repeat expansion in *C9orf72* was performed by geneticists for the MND DNA Bank, Trinity College Dublin.

30 or more hexanucleotide repeats on the *C9orf72* gene is generally considered pathogenic^{66,67}, with others recommending the threshold for pathogenicity be set to 24 and above.⁶⁵ Relatives were deemed positive for the repeat expansion if they had 30 or more repeats (C9+). There is evidence of association between intermediate repeats and ALS, FTD and neuropsychiatric symptoms.^{64,540,541} Therefore, participants with an intermediate expansion (20-29) were also deemed *C9orf72*-positive-carriers in this study. Participants with 19 or fewer repeats were categorised as *C9orf72*-negative (C9-). Repeat length was not available for analysis for individual participants.

In-house genetic testing for research stopped during Covid-19. *C9orf72* status for prospective relatives and controls in Phase 2 of the MND Family Study was therefore not available at the time of writing. Most pwALS in this study underwent clinical genetic testing. If research genotyping data were not available, commercial and clinical genotyping for *C9orf72* was obtained through the Irish National MND Register (described previously by O'Toole et al.⁴⁶).

4.5.1.1 Blinding to genetic status

To maintain strict confidentiality and reduce bias, the study observed double blinding of genetic status for unaffected relatives of pwALS. Geneticists conducting the assays were blinded to participant identities. To ensure all researchers remained blinded to participant genetic status throughout the duration of the study, it was only appended to the database by the ALS research manager after data had been codified.

To facilitate sample enrichment in early stages of the longitudinal research design (MND Family Study Phase 2), a former researcher on the project (EC) carried out purposive

sampling of the initial cohort to include equal numbers of *C9orf72* carriers and non-carriers from fALS kindreds. This maintained blinding of genetic status to the current researchers.

4.5.2 Genetic testing: PwHD

Genetic testing for participants in the HD-Cognition study was not performed. Categorisation of the premanifest group therefore relied on reports from clinical genetics deeming the participant a carrier of the pathogenic expansion (CAG ≥ 39). Repeat length was recorded for pwHD, but this was not always available, particularly for participants who received results many years ago. These carriers were noted in the clinical genetics report as “gene positive with repeat numbers in the pathological range”. Researchers were not blinded to genetic status or number of repeats in the HD-Cognition study.

A limitation of the requirement for clinical genetic testing for categorisation is that it creates a cohort of participants who are at-risk, but do not know their *HTT* genetic status (denoted “Genotype Unknown”). Data from this cohort were collected, as they were willing to participate as a member of a HD family, but were categorised separately. Updates were obtained regarding their genetic status throughout the study and all Genotype Unknown participants were contacted in October 2024 to confirm study group for the final analysis. If genetic status was known, they were reclassified accordingly (premanifest or gene negative control).

4.6 Materials

The neuropsychological assessments were designed to assess executive functions, governed by the dorsolateral-prefrontal, anterior cingulate and medial prefrontal cortices, which underlie much of the cognitive and behavioural functions impaired in ALS and HD.^{332,527} Below is a detailed description of cognitive and behavioural assessments employed. **Table 6** describes each test under the particular cognitive/behavioural domain it falls with reference to the study it is administered in.

4.6.1 Background & demographic questionnaire data

A demographic questionnaire was created on Qualtrics to capture demographic data, family history data and relevant clinical information. It was completed by each the participant in advance of the study appointment. A semi-structured clinical interview supplemented the questionnaires at the time of the neuropsychological assessment to

ensure comprehensive data capture. Demographic data included the participant's date of birth, sex, education (years in formal education and highest qualification), occupation and marital status. Information about any neurological and psychiatric conditions in their own medical and family history was also captured. Additional clinical information included medication use, units per week of alcohol intake, history of diabetes mellitus, hypertension or hypercholesterolemia, history of head trauma or exposure to heavy metals and any history of diagnosed learning difficulty (e.g.: dyslexia). Clinical and demographic data were recorded on the interview schedule form.

4.6.1.1 *ALS-related clinical information*

For pwALS, clinical data were obtained from the Irish MND Register, which also supported confirmation of family history and genetic status of patient probands for relatives within the MND Family study. If register data conflicted with participant reports and could not be corroborated by an additional relative in the same family, data from the register were given preference. These data are collected routinely in the National MND Clinic at Beaumont Hospital to a rigorous standard by a dedicated register manager, ensuring high data quality. Patients consent to inclusion on the national register at their first appointment within the service. Procedures for the MND Register have been previously described.^{46,542-544}

4.6.1.2 *HD-related clinical information*

Clinical data for HD patients was obtained by chart-review and from the local Enroll-HD study site. For those who were not patients of Beaumont Hospital, clinical information was sought from the participants GP/Neurologist via the patient/caregiver. A letter, approved by Beaumont Hospital Research Ethics Committee, was given to the patient to facilitate this transfer.

4.6.2 *Neuropsychological assessment*

The extended batteries of neuropsychological tests were compiled based on the extant literature for domains and tests which were most sensitive to early changes in HD and characteristic of ALS/bi respectively (**Table 6**). They each assessed cognition more broadly to account for heterogeneous presentations, but focused on verbal fluency, various aspects of executive function and social cognitive function, as well as language, memory, and speed of processing. The ALS study battery has been previously validated in an ALS patient population.² Neuropsychological test selection, administration and scoring and

supervision was performed by Principal Clinical Neuropsychologist and PhD Supervisor (NP). Measures used to assess each cognitive domain of interest are described below. Test-specific normative data conversions are described in the relevant section. Scores were converted to standard scaled scores, T-scores and/or z-scores based on published normative data or normative data collected locally. Where possible, these were stratified by education and/or age.

4.6.2.1 Intellectual functioning

The Test of Premorbid Functioning-UK (TOPF-UK)⁵⁴⁵ assessed a participants estimated premorbid level of intelligence. Participants are asked to read aloud a list of 70 words, the pronunciation of which increase in difficulty as the test progresses. A rubric with accepted phonetical structure of each word is used to correct the responses. Participants are afforded leniency if mispronunciations are congruent with their accent. The test is discontinued if a participant mis-pronounces five consecutive words. Raw scores (out of 60) are entered into a program which calculates a predicted Full-Scale IQ (FSIQ) standard score based on their age, education and sex.

The Matrix Reasoning and Vocabulary subtests of the Wechsler Abbreviated Scale of Intelligence 2nd Edition (WASI-II) were used to estimate current intellectual function.⁵⁴⁶ Raw scores are converted to T-scores based on aged matched normative data, which are combined to give an estimate of current functioning, FSIQ-2.

The Vocabulary subtest assesses crystallised intelligence, word knowledge and verbal concept formation.⁵⁴⁶ Participants are asked to define up to 31 words which increase in difficulty as the test progresses. Correct answers are those which match the possible definitions provided in the manual, scored as two points. Participants get one point if they partially define the word, and 0 if they do not meet the minimum criteria for a point. Some 0- and 1-point responses warrant follow up prompt from the examiner, but only for those detailed in the manual.

The Matrix Reasoning assesses fluid intelligence, visual reasoning ability and perceptual organisation.⁵⁴⁶ This task presents participants with up to 30 incomplete patterns which they must complete by selecting one of five options. Difficulty increases with successive trials. Both tasks are discontinued following three consecutive 0-point answers.

4.6.2.2 Social cognitive function

The Reading the Mind in the Eyes Test (RMET)⁵⁴⁷⁻⁵⁴⁹ and Advanced Clinical Solutions (ACS) Social Perception battery (ACS-SP)⁵⁵⁰⁻⁵⁵² were used to measure social cognitive

functioning. The RMET has shown utility in ALS⁵⁴⁸ and other disorders, and short forms (A and B) have been validated in Ireland.⁵⁴⁹

The RMET assesses affective Theory of Mind (ToM), the ability to infer the emotions of another. 36 black and white pictures of the eye region (nose to forehead) displaying complex emotions are displayed in sequence. Participants select the most appropriate mental state from four options, one in each corner. To minimise the effect of language ability, participants are instructed throughout that words can be defined for clarity, if needed. Participants completed either the full or short version with total scores converted to z-scores based on published Irish control data.⁵⁴⁹

The ACS-SP consists of three subtests—Affect Naming, Prosody-Face Matching and Prosody-Pair Matching. The Affect Naming subtask asks participants to choose from a list of 6 emotions, and ‘Neutral’, which best describes the expression shown on an actors face (Happy, Sad, Anger, Afraid, Surprise, Disgust). They are shown 24 images, with the list of seven options displayed concurrently for a maximum score of 24. This task assesses a participants ability to recognise basic facial affect.⁵⁵⁰

The Prosody-Face Matching subtask requires participants to listen to a track of a person making an affectively intonated statement over a blank screen. They are subsequently presented with a grid of six faces, displaying different emotions and instructed to choose the face (numbered 1-6) which best matches the emotion or tone of the speaker’s voice. There are 12 trials in this subtest assessing a participants’ prosodic affect recognition ability. Maximum score is 12, one point for each correct answer.

The 12 trials of the Prosody-Pair Matching subtest followed a similar procedure as Prosody-Face Matching, with additional steps in each trial. After listening to the affectively intonated statement over a blank screen, participants are shown four pairs of actors interacting and must match the emotion or tone of the speaker’s voice with the pair displaying congruent body language and facial expressions. Participants then describe the emotion or tone in their own words, and subsequently determine if the tone of voice altered the meaning of the statement (i.e.: did they mean what they said, or did they imply something else). If participants determined the meaning had changed (true for 6 of the 12 statements), they then described in their own words, what the participant meant. This subtest has a maximum raw score of 42 (12 for prosody-pair, 12 for emotion / tone identification, 12 for meaning change, 6 for speaker meaning if changed).

For each of the ACS-SP subtests, raw scores are converted to scaled scores from standardised and demographically-adjusted norms. Scaled scores are then converted to z-scores (scaled score mean = 10, SD = 3).

4.6.2.3 Processing speed

The symbol digit modalities test (SDMT) is a measure of psychomotor processing speed.⁵⁵³ The written version of the task requires coordination between fine-motor writing speed and cognitive processing speed to match nine symbols with their corresponding number (1-9), displayed in a legend at the top of the page. Symbols are arranged 15 per line on a page, each with an empty box below. Participants must write the number in the boxes in order from left to right and down to the next line. Total score and dependent variable is the number symbols correctly assigned their corresponding number in 90-seconds. Participants complete a 10-item practice, following a 3-item demonstration from the examiner prior to the main trial.

The oral version of this task uses the same stimulus, but asks the participant to call out the corresponding number, instead of writing it down. This removes the confound of limb-motor disability, although still may be affected by motor-speech function. Strober and colleagues⁵⁵⁴ produced normative data for the oral version stratified by age, years of education (<16/≥16 years) and sex, which are converted to z-scores.

4.6.2.4 Executive function

Executive function is an umbrella term encapsulating the processes which direct and facilitate goal-directed behaviour. This includes “initiation, planning, purposive action, self-monitoring, self-regulation, volition...inhibition and flexibility (shifting)” (Stuss, 2011, p. 759)³³², and other constructs such as working memory and attentional control.^{123,555,556} Executive function used to be synonymous with the frontal lobes of the brain, and ‘dysexecutive’ and ‘frontal’ syndromes were often used interchangeably. This domain is therefore particularly relevant in disorders causing fronto-striatal dysfunction, such as HD and ALS. The current studies use a number of different tasks to assess constructs of the executive functions associated with many key associative areas in the frontal- and prefrontal cortices, including the DLPFC, mPFC, inferior frontal gyrus, ACC, and the orbitofrontal cortex.^{142,329,332,523,557}

4.6.2.4.1 Working memory

Working memory is the ability to hold and manipulate information in short term memory. The primary tasks of working memory in this thesis were two of the three condition in the Digit Span subtest of the Wechsler Adult Intelligence Scale 4th Edition (WAIS-IV)⁵⁵⁸, Digit Span Forwards and Backwards (DSF/B). The Digit Span Sequence subtask is also used in the MND family study battery.

The Digit Span tasks, a string of numbers are presented aurally to the participant in a series of trials. The length of the string increases with completion of two trials of the same length. In the Forwards condition, a test of simple attention and auditory sequential processing, the participant must repeat the numbers back to the examiner in the order they were called out. In the Backwards condition, the participant must recall the numbers in reverse order, a more complex task of mental manipulation, which also requires encoding, rehearsal and self-monitoring. Raw scores are the total number of recollections in the correct order (one point per correct response) as well as the longest span of digits. Longest span was converted to z-score based on age-stratified normative data.

4.6.2.4.2 Executive control, inhibition & mental flexibility

The Colour Colour-Word Inference Test (CWIT) from the Delis-Kaplan Executive Function System (D-KEFS)⁵⁵⁹ is a four-condition task that combines multiple executive constructs including inhibitory control, error monitoring, selective attention and cognitive flexibility based on the original Stroop Task.⁵⁶⁰ Speed and accuracy are traded off for successful performance on these four conditions (1-4): Colour-Naming, Word-Reading, Inhibition, Inhibition/Switching.

In each condition, participants are presented with a grid of five rows of 10 items. squares of colour, or the words printed, “red”, “blue” and “green”. They are timed, and must proceed from left to right, naming the colour or reading the word depending on the instructions of that condition, as quickly as possible until they reach the end of the fifth line. There are two class of error which can occur across the trials, self-corrected, when an error is made but it is quickly corrected, and uncorrected errors, when the error is not realised or corrected and the participant continues to the end of the condition. Participants are instructed to balance speed and accuracy. Lower times and fewer errors indicate better performance. Each condition has a practice trial of two lines of five stimuli. The examiner offers feedback to ensure the condition is understood and performance is suitable. If necessary, the practice trial can be repeated. If the participant cannot complete the practice trial, the condition is discontinued.

In Condition 1 (Colour-Naming) participants must name aloud the colour of each of the 50 rectangles, in sequence. In Condition 2 (Word-Reading), participants read a word printed in black ink, aloud in a similar fashion. In Condition 3 (Inhibition), the words are presented in conflicting coloured ink, for example, the word “blue” is printed in red ink. Participants must name the ink colour aloud, “red”, as quickly as possible when the examiner says go, in sequence from left to right and down to the next line, until the end of

the grid. Condition 4 (Inhibition/Switching) is similar to Condition 3 where all words are printed in incongruent ink colours. Participants name the ink colour of each stimulus, not read the word. However, the additional rule is that some of the incongruently printed colour-words have a box around them. If the word has a box around it, participants are instructed to read the word, and not name the ink colour, flipping the rule.

Colour-Naming and Word-Reading (Conditions 1 and 2) are measures of speed of processing and simple cognitive speed respectively. They also provide contrasts and differential controls for the higher level subtests. Condition 3, requires participants to engage in inhibition of the more automatic response—reading the word. Condition 4 also necessitates inhibition of the dominant response, and additionally requires set-shifting and mental flexibility for participants to switch from inhibiting to allowing the automatic response to correctly read the word.

All conditions are scored by converting time to completion and number of errors in each condition to Scaled Scores from published age-based normative data.⁵⁵⁹ Contrast scores included in the analyses isolated the cognitive processing speed of colour naming by obtaining the average of scaled score of Condition 2 and Condition 1, as per published norms.⁵⁵⁹ To isolate the capacity for executive inhibition, the scaled score for processing speed (Condition 1) was subtracted from the scaled score for the inhibition condition (Condition 3), which again provided a scaled score based on normative data.⁵⁵⁹ The average scaled score of Condition 1 and 2 was subtracted from that of Condition 4 to give a participants capacity for inhibition and switching together. Lastly, the final contrast score subtracted Condition 3 from Condition 4 to give a measure of switching capacity. All scaled scores were subsequently converted to z-scores for analysis.

4.6.2.5 Verbal fluency

Verbal fluency requires a complex interplay of executive and linguistic processes relating to generation and switching, and retrieval from the mental lexicon.^{137,138} The current studies employed three verbal fluency paradigms: phonemic, semantic and restricted-phonemic fluency. In all conditions, participants are given 60 seconds to generate aloud as many words as possible as quickly as possible, meeting the stipulations of that condition / trial set by the examiner. Two types of errors are possible. Set-loss errors occur when participants provide a word that do not meet defined criteria. Repetition, or perseverative errors, are when participants provide words that they have already said earlier in the trial. Each condition/trial has a total raw score of correct responses within the 60-second window.

4.6.2.5.1 Verbal Fluency Index

After each verbal fluency condition, correct responses are transcribed and participants are asked to read them aloud as quickly as possible. The examiner records the time taken to read the words aloud in order to calculate the Verbal Fluency Index (VFI).¹¹⁸ The VFI accounts for motor impairment, and is therefore particularly relevant disorders affecting the motor system. It provides a truer estimation of cognitive function, minimising the confounding effects of motor function.¹¹⁸ It is calculated as follows:

$$VFI = \frac{60 - \text{reading time}}{\text{number of correct responses}}$$

4.6.2.5.2 Phonemic verbal fluency

Phonemic verbal fluency requires recruitment of higher order processes of executive function (i.e.: initiation, updating, inhibition, monitoring) and language, as well as phonological and lexical processes within working memory. It is a particularly sensitive marker of cognitive dysfunction in ALS.¹¹⁸

The FAS task is a version of the Controlled Oral Word Association Test requiring participants to produce words beginning with the letters “F”, “A”, and “S” as quickly as possible within the time frame.^{559,561} Criteria for this task is that words must not be the “names of people, names of places, or numbers”. Words meeting these criteria, were marked incorrect, as a set-loss error. Total correct responses are converted to z-scores based on age and education stratified normative data.⁵⁶¹ Repetition errors are also recorded.

4.6.2.5.3 Restricted phonemic verbal fluency

The restricted fluency task asks the same of participants as described above, with the additional restriction of correct words must only be four letters long. The current study used the letter “C”. There are no published normative data for this task, and so the VFI was used in analyses. The mean and standard deviation of locally generated control data were then used to convert the VFI to z-scores.^{118,175}

4.6.2.5.4 Semantic fluency

Semantic fluency, also referred to interchangeably as category fluency, requires many of the executive and language processes necessary for phonemic fluency, but is an easier task underwritten by semantic processes of the temporal lobe.¹⁴⁵ Task conditions require participants to name as many animals as possible. Published manual protocols

were adhered to.⁵⁵⁹ Total correct responses are converted to z-scores based on age and education stratified normative data.⁵⁶¹

Semantic fluency has many components which often classify it as a task of executive function, but it is also occasionally grouped with the language tasks due to its reliance temporal semantic networks and lexical-semantic memory.¹⁴⁵

4.6.2.6 Language

The Sydney Language Battery⁵⁶² assessed language function in the HD-Cognition study, with the Boston Naming Test (BNT) measuring confrontation naming performance in the MND Family Study.⁵⁶³

Confrontation naming is a task primarily of word retrieval with semantic knowledge requirements.⁵⁶⁴ The 30-item version of the BNT asks participants to name a series of black and white line drawings, one at a time, which increase in difficulty.⁵⁶⁵ If a response is not provided spontaneously, i.e.: the participant cannot name the object shown, they are prompted with a semantic cue, providing additional information about the use or context of the object. Failing that, they are lastly offered a phonemic cue, i.e.: “the word begins with the sound...’x’”. Total spontaneous responses converted to z-scores from age-matched locally validated normative data for analyses.

The Sydney Language Battery comprises three sub-tests assessing different domains of language function.⁵⁶² 30 full-colour pictures of objects increasingly less common/more difficult, are presented in series. Subtests include a confrontation Naming subtask, similar to the BNT described above, a single-word Comprehension subtask and a Semantic association subtask, presented in that order. The single-word Repetition subtest is not used in this study. Stimuli are consistent across subtests (e.g.: elephant), with alternative pictures used in subsequent subtests to minimise visual memory effects. Total score on all subtests is the number of correct answers out of 30.

In the Naming condition, participants are shown individual colour photographs and asked to name each item aloud. The examiner says the target word aloud in the Comprehension subtask. Participants then select the correct item from an array of seven numbered photographs. Foils are either semantically or visually similar to the target. The Semantic Association subtest instruct participants to choose the most semantically related image from a set of four options below the target image. Distractors are semantically related to each other but unrelated to the target.

4.6.2.7 Memory

There are three domains of memory assessed in these studies: Verbal list learning, verbal story memory and visual memory. In each, there are encoding, or learning trials, immediate recall of the information, a delayed recall condition and a recognition condition.

Two verbal list-learning tests were used; the Rey Auditory Verbal Learning Test (RAVLT)⁵⁶⁶ in the MND Family Study and the California Verbal Learning Test 3 (CVLT-III) in the HD-Cognition study.⁵⁶⁷ They have similar structure and have been shown to assess comparable constructs of verbal learning and memory, particularly for the learning, immediate recall, delayed recall and recognition trials.⁵⁶⁸ In a clinical sample with multiple sclerosis, Beier et al. (2019)⁵⁶⁹ demonstrated similar diagnostic accuracy between the instruments. The CVLT-III's distinguishing feature are cued recall trials (immediate and delayed), and manualised procedures for scoring strategic encoding patterns, and intrusion and error analyses. These scores aid in distinguishing primary memory impairments and deficits in retrieval strategies or inhibitory control, which are hallmarks of frontostriatal dysfunction in HD.^{570,571} Published procedures for the CVLT-III⁵⁶⁷ and the RAVLT⁵⁶⁶ were followed in all studies.

For the RAVLT, participants were read a list of 15 unrelated words (List A) and asked to recall as many items as possible immediately following presentation. This was repeated over five consecutive learning trials, yielding a maximum total score of 75 across the five list-learning trials. A single interference trial (List B) was then presented, followed by an immediate recall of the original List A (post-interference recall). After a 30-40 minute delay, participants completed a delayed recall trial, aiming to recall as many words as possible from List A. A recognition trial is then administered asking participants to identify List A words from a 50-word list that included distractors (i.e.: words from List B, as well as semantically and phonemically related foils). Raw scores were converted to z-scores using age- and sex-adjusted normative data.⁵⁷²

The CVLT-III follows similar structure, with 16 unrelated words presented in the five learning trials, and another 16 in the distractor list (List B). Semantic clustering is possible in the CVLT trials, as words fit into four broad semantic categories. Following immediate recall trial after the distractor list, participants are asked to provide any words they can recall from the list that fit into a prescribed category, provided in turn. The four categories for cued recall trials are “Furniture”, “Vegetables”, “Ways of Travelling” and “Animals”. Delayed free and cued recall trials are administered after a 30-40 minute interval. A yes/no

recognition trial that includes target words, distractors, and semantically/phonemically similar foils follows this. Performance was scored using standardised procedures. Raw scores (trial totals) were converted to scaled scores and subsequently to z-scores based on age-adjusted normative data.⁵⁶⁷ Additional variables of interest for the CVLT was the number of errors in free- and cued-recall trials and the non-parametric discriminability index from which a scaled score is calculated reflecting a participant's ability to accurately differentiate targets from distractors.

4.6.2.7.1 Story memory

Logical Memory assesses episodic verbal memory, encompassing encoding, storage, and retrieval processes. Its narrative format engages higher-order functions including conceptual organisation and schema-based memory. Stories B and C from the Wechsler Memory Scale 4th Ed. (WMS-IV)⁵⁵⁰ assess verbal learning and recall for short stories. Successful completion of these trials requires attention, encoding, recall and recognition of verbal story information. Participants listen to the two aurally presented stories and subsequently asked to recall as much details as possible, using similar language, immediately following its presentation (immediate recall). After a 30-40 minute delay, participants were again asked to recall the two stories, one at a time. Following the delayed recall trials, participants completed a recognition task consisting of 15 true/false questions per story. Performance was scored according to standardised criteria and converted to z-scores using age-adjusted published normative data.⁵⁵⁰

4.6.2.7.2 Visual memory & visuo-constructive ability

Visuospatial memory was assessed using the Rey-Osterrieth Complex Figure Test (RCFT). This standardised task requires attention, organisational strategy, visual perception as well as encoding, storage and retrieval of detailed visual information.⁵⁷³. Copy score and time to copy are also measures of visuospatial construction.

Participants are instructed to copy a complex geometric figure as accurately as possible (copy trial), with the time taken to complete the drawing recorded. The figure is removed, and participants asked to reproduce it from memory immediately after (immediate recall). Following a 30-40 minute interval, a second reproduction is requested from the participants memory (delayed recall). Participants then complete a recognition trial in which they are shown 24 structural elements, 12 from the original figure and 12 distractors, and asked to identify the original components (yes/no).

Scoring of the Copy and subsequent recall trials is based on 18 distinct figure elements. Each component was scored from 0 to 2. 2 points for accurately drawn and

correctly placed elements; 1 point for either accurately drawn but misplaced or inaccurately drawn but correctly placed elements; 0.5 points for elements that were poorly drawn and misplaced but still recognisable; and 0 points for missing or unrecognisable components. Raw scores were standardised to z-scores using published age-adjusted normative data.⁵⁷³

4.6.3 Cognitive screening: the ECAS

The Edinburgh Cognitive ALS Screen (ECAS) is a repeatable, multidomain cognitive screener with three serial forms; A, B and C.^{159,162,163} It is composed of 5 subdomains, three of which fall under an ALS-Specific domain score (Language, Verbal Fluency and Executive Function), and two which combine to give an ALS-non-Specific score (Memory and Visuospatial function). This division reflects the established pattern of cognitive change in ALS, with ALS-Specific domains considered particularly vulnerable, while Memory and Visuospatial abilities are relatively preserved.¹⁵⁹ The ECAS Total score is the sum of both composite scores. Clinical cut-off scores for four demographic categories have been created and validated in the Irish population for each domain score according to age and years of education (≤ 65 / ≥ 12 years of education; **Appendix 6**).^{162,163} The ECAS is well-established as a sensitive screening measure, capable of identifying cognitive impairment even in patients with physical disability, and has demonstrated utility across both clinical and research contexts. Its use has been proposed for the categorisation of extramotor impairment under the Strong Criteria^{23,574}, and is a secondary endpoint measure in clinical trials in ALS.

4.6.4 Administration & scoring

The neuropsychological assessment was performed in accordance with published manual guidelines. Instructions were given to participants at the start of every task and clarification for understanding was encouraged. Raw scores were converted to T-scores, z-scores, standard scores or scaled scores, whichever conversion was provided in test manuals. z-score conversion from education- and/or age-stratified normative data was preferred. Scaled scores were converted to z-scores for consistency across measures. All study data were stored in password protected Microsoft Excel databases.

Questionnaires were administered online using Qualtrics. Demographic information was printed off and stored with paper files. Scoring of neuropsychological measures (behaviour/mood/function) was pre-coded by the PhD student (CP). Data were

stored on the Qualtrics server before being exported and stored in password protected Microsoft Excel databases. Domain and scale total scores were calculated for analysis, and published cut-off scores categorised abnormality/impairment.

Table 6: Details of extended neuropsychological test battery: cognitive assessment.

Test	Subtest(s)	Cognitive function assessed	Project
Screening			
Edinburgh Cognitive and Behavioural ALS Screen (ECAS) ¹⁵⁹		Screening tool of language, executive functioning, verbal fluency, visuospatial ability and memory	3
Premorbid and Current Intellectual Function			
Test of Pre-morbid Function (TOPF). ⁵⁴⁵		Pre-morbid intellectual functioning	3
Wechsler Abbreviated Scale of Intelligence, 2 nd Ed. (WASI-II) ⁵⁴⁶	Vocabulary	Semantic knowledge, verbal comprehension and expression	3
	Matrix Reasoning	Nonverbal abstract problem solving and inductive reasoning	
Cognitive Reserve Index Questionnaire (CRIQ) ⁵⁷⁵		Structured quantitative interview to estimate cognitive reserve based on educational and professional attainment and leisure activities	2 ^a
Executive Function			
Verbal Fluency / Verbal Fluency Index (VFI) ^{118,559,561}	Phonemic (FAS) Category (Animal) Restricted fluency (Letter C)	Series of lexical and semantic fluency tasks with defined criteria	3
Colour-Word Inference Test (D-KEFS). ⁵⁵⁹	1. Colour Naming 2. Word Reading 3. Inhibition 4. Inhibition/Switching	Measures multiple dimensions of executive control, including inhibitory control, error monitoring, selective attention and cognitive flexibility. First two subtests are also proxy measures of speed processing	3
Digit Span. ⁵⁵⁸	Forward Reverse Sequence	Simple attention and working memory	3
Sustained Attention to Response Task (SART). ⁵⁷⁶		Sustained Attention	1
Iowa Gambling Task (IGT). ⁵⁷⁷		Emotion-based decision making and probabilistic learning	1
Social Cognition			
Reading the Mind in the Eyes Test (RMET). ⁵⁴⁷⁻⁵⁴⁹		Affective theory of mind	3
Advanced Clinical Solutions (ACS). ⁵⁵⁰	Social perception battery (ACS-SP)	Emotion recognition (facial expression and prosody) and affective theory of mind	2
Psychomotor Processing Speed			

Test	Subtest(s)	Cognitive function assessed	Project
Symbol Digit Modalities Test. ^{553,554}		Psychomotor processing speed	3
Language			
Sydney Language Battery. ⁵⁶²	Confrontation Naming Comprehension Semantic Association	Word retrieval, auditory comprehension and semantic information for nouns	2
Boston Naming Task: 30-word version (BNT). ⁵⁶³		Confrontation naming task; measures word retrieval and semantic knowledge for nouns	1
Memory			
Wechsler Memory Scale 4 th Ed. (WMS-IV). ⁵⁵⁰	Logical Memory, Story B & C	Attention, encoding, recall and recognition of verbal story information	3
Rey Auditory Verbal Learning Test (RAVLT). ⁵⁶⁶		Attention, encoding, recall and recognition of verbal list information	1
California Verbal Learning Test III (CVLT-3) ⁵⁶⁷		Attention, encoding and recognition, and free- and cued-recall of verbal list information	2
The Rey Complex Figure Test (RCFT). ⁵⁷³		Visuospatial and constructive ability, and visuospatial memory	3
<i>Project code: 1 = MND Family Study only; 2 = HD-Cog only; 3 = HD-Cog and MND Family Study; ^a structured interview format. ^b PwHD only.</i>			

4.6.5 Behavioural assessment

Neuropsychiatric and behavioural assessments were completed online in advance of the main neuropsychological assessment appointment. In instances where participants did not complete them in advance, they and their companions were given 7-days to complete the questionnaires following the assessment. Data submitted outside of this window were not included in analyses. The study procedures are outlined in subsequent chapters and in **Appendix 3**. Questionnaires and the particular studies employed are detailed in **Table 7**.

4.6.5.1 Behavioural screening

The Beaumont Behavioural Inventory (BBI)^{153,578}, Frontal-Systems Behavioural Scale (FrSBe)⁵⁷⁹ and Dimensional Apathy Scale (DAS) were completed by participant companions as a measure of behavioural function. The self-rated version of the FrSBe and DAS are also completed by the participant.

The BBI is a 41-item self-explanatory proxy-rated measure for the assessment of behavioural impairment, with a particular focus on domains relevant to ALS and FTD.^{153,578} It is designed to capture a broad spectrum of behavioural symptoms over two time-frames pre- and post-symptom onset, while accounting for confounding effects of neurodegeneration associated motor impairment. For symptomatic pwALS and PwHD, the

standard version was used, with changes to wording in the instructions for HD participants about HD-symptom onset. For control and premanifest HD participants, and the relatives of pwALS in the MND Family Study, a control version was administered, which asked about change over the past 10 years and in the past 3–5 years. Each item is rated on a 4-point scale: ‘No change’ (0), ‘Mild change’ (1), ‘Moderate change’ (2), or ‘Severe change’ (3). Scores across all items are summed to produce two total BBI scores; a) pre-symptom onset / past 10 years and, b) post-symptom onset / past 3-5 years.

Published clinical thresholds were applied to categorise behavioural change.^{153,578} Scores of less than seven on the BBI indicate no behavioural change and between 7 and 23 denote mild-behavioural change. 23 and above indicate severe behavioural change and have high sensitivity and specificity for bv-FTD.⁵⁷⁸ The BBI has shown superior diagnostic accuracy compared to other ALS-specific behavioural assessments.¹⁵³

The FrSBe is a 46-item questionnaire designed to assess changes in behaviour associated with frontal lobe dysfunction.⁵⁷⁹ Both self-rated and informant/proxy-rated versions were administered. Respondents rate the frequency of behaviours on a 5-point Likert scale ranging from 1 (‘Almost never’) to 5 (‘Almost always’). As with the BBI, for manifest HD participants the standard version was administered, asking respondents to rate behavioural frequency both prior to symptom onset and after symptom onset (both time periods for each of the 46 items). For control and premanifest HD participants and unaffected relatives of pwALS, a modified version was used, asking them to consider behaviour 3–5 years ago, and in the present. This allowed for the assessment of perceived behavioural change over time in both clinical and non-clinical groups.

4.6.5.1.1 *Apathy*

The Dimensional Apathy Scale (DAS) is a 24 item self-report questionnaire with self-report and informant-reported versions.⁴³² Created to minimise the effects of motor dysfunction, particularly in ALS, respondents are asked to rate the frequency at which statements are true on a 4-point Likert scale (Almost always, Often, Occasionally, Hardly Ever). Some items are reverse scored, so that higher domain scores are infer greater levels of apathy. Scores are calculated according to published guidelines, producing total scores across three domains: Emotional, Executive and Behavioural/Cognitive Initiation Apathy.⁴³² Published control data sets clinical cut-off scores in each domain, with combined scores over 39 indicative of clinically significant Total Apathy.¹⁵⁴

4.6.6 Neuropsychiatric assessment

4.6.6.1 Mood

A modified version of the Hospital Anxiety and Depression Scale (HADS-R)⁵⁸⁰ was used to screen for mood disturbance and psychological distress. This adaptation omits items that may be confounded by physical disability, such as those referring to psychomotor slowing, appearance, and restlessness (items 8, 10, and 11, respectively). The scale can be broken into anxiety and depression domains. It is validated for pwALS, demonstrating strong construct validity, internal consistency, and reliability.^{580,581} Revised clinical cut-offs are applied so that total scores of 17–20 indicate a possible mood disorder, while scores of 21 or above suggest a probable mood disorder.⁵⁸⁰

In different aspects of the studies, mood symptoms were assessed using the PHQ-9⁵⁸² and GAD-7.⁵⁸³ The PHQ-9 evaluates the presence and severity of depressive symptoms based on DSM-IV criteria, with each item rated from 0 ('Not at all') to 3 ('Nearly every day'). Total scores categorise symptom severity as follows: 0–4 (none), 5–9 (mild), 10–14 (moderate), 15–19 (moderately severe), and ≥ 20 (severe). A cut-off score of ≥ 10 has demonstrated 88% sensitivity and specificity for major depressive disorder.⁵⁸²

The GAD-7 assesses symptoms of generalised anxiety over the past two weeks using a similar 0–3 response scale. Total scores are interpreted as follows: < 5 (minimal), 5–9 (mild), 10–14 (moderate), and ≥ 15 (severe), with a threshold of ≥ 10 yielding high sensitivity and specificity for generalised anxiety disorder.⁵⁸³

4.6.6.1.1 Caregiver questionnaires

All participants, including controls were requested to have someone who knows them well complete informant-rated behavioural questionnaires (BBI, FrSBe and DAS). Caregivers/companions of PwHD participants were additionally asked to complete questionnaires assessing caregiver burden, strain, wellbeing and coping strategies for the *HD-Carer study*, which runs concurrently within HD-Cognition (**Appendix 10**). These data are not reported here. Qualitative data collected in this study have recently been published.³⁹⁴

4.6.7 Functional assessments

4.6.7.1 Assessment of motor function for pwALS

The Revised ALS Functional Rating Scale (ALSFRS-R)⁴² is a 12 item measure of motor function impairment in ALS. It is typically completed by a clinician with a pwALS

quantifying dysfunction across bulbar, spinal and respiratory symptoms. It includes three items assessing bulbar function (speech, salivation, and swallowing), six items evaluating motor function—subdivided into fine motor tasks (handwriting; cutting food and handling utensils) and gross motor tasks (dressing and hygiene; turning in bed and adjusting bedclothes; walking; and climbing stairs)—and three items assessing respiratory function (dyspnoea, orthopnoea, and ventilatory support). Each item is scored from 0 to 4, with higher scores indicating better functional ability. Total scores range from 0 (complete functional dependence) to 48 (no disability).

4.6.7.2 Functional assessment & wellbeing in HD-Cognition

All participants in HD-Cognition completed questionnaires of functional status, health, wellbeing and quality of life (QoL), as per study procedures below. Scales from the UHDRS, HD-specific measures of functional ability, were completed with PwHD participants only at the beginning of each study appointment.

4.6.7.2.1 The Unified Huntington's Disease Rating Scale

Functional status and independence in HD was assessed using three subscales from the UHDRS²⁸⁸: Functional Assessment, Total Functional Capacity (TFC) scale, and the Independence Scale, each capturing different aspects of everyday functioning. This is administered as a structured interview with PwHD.

The Functional Assessment scale is a 25-item checklist of common daily activities, including household tasks, personal care, mobility, and community participation. For each activity, the assessor records whether the participant is able to complete the task independently (yes/no). Total score is derived by summing all “yes” responses. Higher scores indicate greater retained functional ability. This checklist provides a granular view of specific abilities lost or preserved in the course of disease progression.

The TFC is a validated five-item clinician-rated scale assessing occupational status, financial management, domestic chores, activities of daily living (ADLs), and care level. Each item is scored on a ordinal 0-2/0-3 scale. Total scores ranging from 0 to 13, lower scores signifying greater functional impairment. The TFC is widely used as a global marker of disease progression and is a central element in HD clinical staging. The TFC is widely used to track disease progression has shown high interrater reliability and sensitivity to changes over time in manifest HD.²³⁵

The Independence Scale quantifies overall functional independence on a scale from 0 to 100, with higher scores indicating greater independence. The scale is anchored at fixed intervals of 5 (e.g.: 100, 95, 90...) and is based on the degree of assistance required

across a range of daily tasks, from employment and financial management to self-care and mobility.

4.6.7.2.2 *General Functional Disability, Quality of Life & Fatigue in HD*

Functional status, health-related Quality of Life (QoL), and fatigue were assessed using a battery of validated self-report instruments, including the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0)⁵⁸⁴, the EuroQoL 5 Dimensions Scale (EQ-5D)⁵⁸⁵, the Fatigue Symptom Inventory (FSI)⁵⁸⁶, and the Multidimensional Fatigue Symptom Inventory – Short Form (MFSI-SF).⁵⁸⁷

The WHODAS 2.0 is a standardised instrument developed by the World Health Organization to measure disability, functional impairment and QoL related to health conditions across six domains: understanding and communicating (cognition), mobility, self-care, getting along with others, life activities (household, school/work), and participation in society. Participants rated the level of difficulty experienced in each domain over the past 30 days (none, 1; mild, 2; moderate, 3; severe, 4; and extreme, 5), summed together to provide a global index of disability.⁵⁸⁴ It has shown excellent psychometric properties⁵⁸⁸, recently recommended by the Neuropsychology Working Group of HSG for use in HD.³¹⁰

The EQ-5D-3L is a widely used measure of health-related QoL (HR-QoL), assessing five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Responses are on a 3-point scale used to derive a single index value reflecting overall health status. The sixth (EuroQoL_6) item is a visual analogue scale on which respondents rate their current health state today on a 0-100 scale, with 100 as best imaginable health, and 0, worst imaginable health, representing a respondents' HR-QoL.⁵⁸⁵

The FSI is a 13-item self-report measure designed to evaluate fatigue intensity, duration, and its interference with daily life over the past week. Respondents rate fatigue intensity (4 items) and interference (7 items) on 11-point Likert scales ranging from 0 to 10. The total score for the interference subscale ranges from 0 to 70, with higher scores indicating greater disruption to QoL due to fatigue. The FSI has demonstrated strong internal consistency ($\alpha > 0.90$) and validity in oncology and across clinical populations, providing a comprehensive profile of subjective fatigue burden.⁵⁸⁶

The MFSI-SF is a 30-item measure of fatigue-related symptoms that assesses the multiple dimensions of fatigue over the previous week. Each item is rated on a 5-point Likert scale ranging from 0 ("Not at all") to 4 ("Extremely"), yielding five subscale scores that together yield a Total Fatigue Score: General, Physical, Emotional, and Mental Fatigue,

along with Vigour. Total Fatigue is the sum of each of the four first domains, from which Vigour score is subtracted, producing a composite index of fatigue severity (range: -24 to 96).⁵⁸⁷ Higher total and subscale scores, reflect greater fatigue, with the exception of Vigour. The MFSI-SF demonstrates strong psychometric properties across cancer and chronic illness populations, with internal consistency values exceeding 0.85 for all subscales.⁵⁸⁷ The FSI and MFSI-SF offer complementary perspectives on fatigue. The FSI provides a brief and sensitive measure of fatigue intensity, duration, and functional interference, capturing both variability across days and perceived disruption to QoL. In contrast, the MFSI-SF offers a multidimensional profile, enabling more nuanced assessment of fatigue across distinct domains.

4.6.8 Other neuropsychological assessment measures

Data collected for the wider MND Family Study, but not included in analyses here included the Iowa Gambling Task (IGT)⁵⁷⁷ and Sustained Attention to Response Task (SART).⁵⁷⁶ Both are computerised tasks requiring specialised software, and must be administered in person. The IGT is a computerised measure of emotion-based decision making, in which participants select cards from four decks with varying reward/punishment profiles, aiming to maximise profit.⁵⁷⁷ Successful performance depends on learning to favour advantageous and avoid disadvantageous decks.

The SART is a brief, computer-based go/no-go task assessing sustained attention and inhibitory control. Participants respond to a stream of single-digit numbers, withholding responses only when the number '3' appears. Performance indices include commission and omission errors, reaction time, and attentional lapses.⁵⁷⁶

4.6.8.1 Neuropsychiatric assessment in the wider MND Family Study

The UK Biobank Thoughts and Feelings Questionnaire^{589,590} was administered to unaffected relatives of pwALS in the MND Family study, some components of which are detailed above and included in the analyses in subsequent chapters: the DAS, PHQ-9, GAD-7. The Rust Inventory of Schizotypal Cognitions (RISC)⁵⁹¹ is a 26-item self-report questionnaire designed to assess the presence and frequency of schizotypal thinking and cognitive features associated with the positive symptoms of psychosis. Participants rate items related to unusual or bizarre thoughts, with total scores ranging from 0 to 78. Higher scores indicate a greater incidence of schizotypal cognitions. Other components of this assessment have been addressed in previous works^{194,210,211} and will not be addressed in the current studies (**Appendix 9**).

Table 7: Details of extended neuropsychological test battery: psychiatric/behavioural assessment.

Neuropsychological test	Function assessed	Project
Mood Questionnaires		
Patient Health Questionnaire 9-item (PHQ-9) ⁵⁸²	Mood disturbance; depression	3
Generalised Anxiety Disorder 7-item Scale (GAD-7) ⁵⁸³	Mood disturbance; anxiety	3
Hospital anxiety and depression scale revised (HADS-R; Version 2) ⁵⁸⁰	Mood disturbance; anxiety and depression	3
Dimensional Apathy Scale (DAS; informant & patient rated) ⁴³²	Apathy in three different dimensions; executive, emotional, and cognitive/behavioural initiation	3
Behavioural Questionnaires		
Beaumont Behavioural Inventory (BBI; proxy-rated) ⁵⁷⁸	41-item Informant-rated scale of general behaviour change pre/post-symptom onset.	3
Frontal Systems Behaviour Scale (FrSBe) ⁵⁷⁹	Self- and Informant-completed behaviour rating scale testing for behaviour change typical of frontal-subcortical circuit disturbance	3
Rust Inventory of Schizotypal Cognitions (RISC) ⁵⁹¹	26-item questionnaire quantifying the cognitive content of participants, created to identify bizarre thinking and the positive symptoms of psychosis.	1
Health & Functional Questionnaires		
General Health Questionnaire: 12 item version (GHQ) ⁵⁹²	Screen of non-psychotic and minor psychiatric disorders	1
World Health Organisation Disability Assessment Scale (WHODAS) ⁵⁸⁴	Measures difficulties associated with health problems in the past 30 days	2
EuroQol 5 Dimensions Scale (EQ-5D-3L) ⁵⁸⁵	Standardised measure of health status and quality of life on 5 dimensions	2
Fatigue Symptom Inventory (FSI) ⁵⁸⁶	14-item self-report measure assessing the severity and frequency of fatigue and the perceived interference with quality of life.	2
Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) ⁵⁸⁷	30-item short form that assesses the main presenting symptoms of fatigue. Contains 5 subscales addressing general, physical, emotional, & mental manifestations of fatigue, as well as an estimate of the patient's energy levels (vigour)	2
UHDRS (Core functional Scales).Huntington Study ²⁸⁸	Structured interview questions on three functional scales: Functional assessment, Independence scale and Total Functional Capacity (TFC)	2 ^{a,b}

Project code: 1 = MND Family Study only; 2 = HD-Cog only; 3 = HD-Cog and MND Family Study; ^a structured interview format. ^b PwHD only.

4.7 Procedures

Following expression of interest in participation, the study was discussed with prospective participants on the phone, and participant information leaflets (PIL) were sent via email/post with instructions to read them thoroughly and to contact the researchers if any questions arose. To allow ample time for consideration, a follow-up phone call (approx. 1 week later) ascertained their willingness to participate after having read the PIL and offered the possibility to discuss any questions. If consenting, a suitable time and date for the study appointment was arranged an online questionnaire and consent form, hosted on Qualtrics, was sent to the participant by email. Participants received copies of both the PIL and the consent form for their own records.

A scheduled email for 7 days before the appointment included webpage links to the neuropsychological questionnaires detailed in **Table 7**. They were instructed that questionnaires should be completed in advance of the scheduled appointment. Links brought participants to an anonymised questionnaire, hosted on Qualtrics, into which they were instructed to enter their study code, also provided in the email. The first link was for the participant to complete questionnaires about themselves. They were instructed to send the second link, along with their study ID, to someone who knew them well who could act as a proxy or informant. Questionnaires took approximately 15-45 minutes, depending on the study and version. In a small number of cases, participants did not wish to complete the online questionnaires. In such cases, a paper version was made available, which participants returned by post.

The majority of the study appointments were carried out online, with a number also being carried out in the participant's home, Beaumont Hospital, or in the Biomedical Sciences Institute, TCD, depending on participant availability and preference. In all settings, appropriate environmental conditions for testing was ensured to minimise outside disruption and maximise participant privacy. The neuropsychological assessment comprised the battery of measures outlined in **Table 6** and **Table 7**. It took approximately 2 to 3 hours to complete. A scheduled 5-10 minute break was provided at the halfway point, with additional breaks offered as needed, to reduce fatigue.

For prospective participants in Phase 2 of the MND Family Study, a blood sample was collected for genetic analysis (see **Section 4.5.1**). This was performed at the end of the assessment, if the appointment was in-person. If online, another appointment was scheduled in order to arrange collection of the blood sample, in accordance with the Covid-19 travel and social distance guidelines at the time.

4.7.1 General neuropsychological assessment procedures

Neuropsychological assessments were performed by the PhD Student (CP) across all studies. Another PhD student also performed assessments in the Pleiotropy/MND Family study, and research assistants working on the HD-Cognition project. The neuropsychological assessment is scored by the administering researcher using the relevant test manuals or published normative data for that test.

To ensure consistency of the data between testers administering the neuropsychological battery, protocols were exchanged, discussed and updated where necessary. Inter-rater reliability studies were carried out for the author (CP) and previous researchers on the MND Family Study, and subsequently with research assistants on both projects. Each tester observed multiple participants. Assessments were scored together and any discrepancies were debated until absolute agreement was achieved. The new tester would then alternate with the senior tester to administer and score consecutive participants. Any variation in administration between testers was discussed and a subsequent process was carried out until absolute agreement was attained in accordance with the manuals. Intra-class correlations (ICC) were carried out for consistency with scoring. Once an ICC of 0.9 or higher was achieved, new testers could complete assessments independently.

4.7.2 Cognitive screening: ECAS procedures

All researchers received training from the creators of the ECAS and followed training guide materials on '<https://ecas.psy.ed.ac.uk/>'. Published locally generated normative data, VFI conversion and guidelines were followed where relevant (**Appendix 7**).^{162,163}

4.7.3 Procedures for remote / online study appointments

Neuropsychological assessment employed in this research would ordinarily be administered in-person by pen-and-paper or computer-based interface, depending on the test. The Covid-19 pandemic affected how these studies could be carried out. In order to allow the studies continue throughout the pandemic, protocols for online administration of the neuropsychological battery had to be created and validated. Stakeholders from voluntary organisations and from the patient community were involved in participant and public involvement (PPI) following creation of these new protocols and stimuli. Described in **Chapter 5**, an online validation study was carried out to ensure that the online

neuropsychological test battery was a valid and reliable alternative to the standard in-person administration. Alongside validation of the materials, protocols were created to manage risk that might arise during testing or interview, and general protocols to ensure consistency between subject settings. Online protocols (**Appendix 2 & 3**) were created by PhD Candidate (CP) and supervised by PhD Supervisor (NP) in accordance with best practice guidelines for teleneuropsychology.⁵⁹³

Online assessments were carried out online using T-Pro (until 2021) or AttendAnywhere (since 2021).Health Service ⁵⁹⁴ The share-screen function allows presentation of stimuli, as they would be from a stimulus book at an in-person assessment. Both platforms meet ISO 13131 standards for telehealth services, providing a secure and reliable virtual platform for remote communication, specific to healthcare and health research.

4.7.3.1 Questionnaire administration & data collection

All studies described in the following chapters employed the use of questionnaires to collect demographic information and to quantify different neuropsychological traits and psychological status (described above). To allow for these to be administered remotely, as the rest of the neuropsychological battery, the pen-and-paper based questionnaires digitised and codified using Qualtrics. The Qualtrics platform is compliant with ISO/IEC 27001 standards for information security management systems.

Participants were given a unique participant study ID code and asked to enter this at the start of any online questionnaires. Results were returned automatically and stored, codified, on the Qualtrics platform, where they were later downloaded for local storage and analysis in password protected Microsoft Excel databases. Each questionnaire contained a blurb reminding participants of the purpose of the questions, the confidential storage of their answers and the limits to this confidentiality (**Appendix 4**). Examiner and study information, and links for further information were displayed at the end of each questionnaire.

4.7.3.2 Online neuropsychological assessment procedures

Cognitive stimuli were displayed to the participant via PowerPoint presentation using the share screen function. Minimum screen size requirements endeavoured to maintain the size and proportions of the stimuli. To ensure the participant's connection and screen size were sufficient, test stimuli from the online version of the ECAS⁵⁹⁵ were presented at first time of screen sharing. Tests not requiring visual stimuli were administered according to standard procedures.

4.7.3.2.1 Requirements for online assessment

Best-practice guidelines for telepsychology were adhered to and shaped protocol creation, which included requirements for screen-size, internet connection and privacy.⁵⁹³ Participants were advised to use a laptop or desktop computer for the assessments. If these were not available, iPad or tablets with a screen size of 9" or larger were acceptable. Use of a smartphone was not permitted. Approximate screen size was taken into account by the examiner when administering the neuropsychological stimuli.

High speed internet was a further requirement to ensure seamless transmission of stimuli and responses, especially pertinent for timed tasks. Participants were instructed to not to record any aspect of the assessment. Clear instructions were given at the start of every assessment that participants should not write anything down, unless explicitly asked to do so. Reminders were also given at each relevant subtask.

4.7.3.2.2 Examiner requirements: remote assessment

The examiner carried out the online assessment using a MacBook Pro 13" (2020). Other examiners used laptops with similar specifications and screen-size. Examiners were always in a private room, without any chance of being overheard or interrupted. No aspect of the online assessments were recorded. However, three screenshots were taken of the RCFT figure drawn by the participants for scoring purposes. The participant was not captured in these screenshots, nor was any other identifiable information. Screenshots of the drawings were added to a Microsoft Word document which were printed out and stored with the rest of the hard-copy response sheets. Screenshots were subsequently deleted.

4.7.3.2.3 The online testing environment

Participants completed the online assessments in their own homes. As a result, researchers did not have as much control over the participant's testing environment as would be the case in a clinical setting. Privacy was important from both a GDPR perspective and to maintain the participant's attention, minimising distraction throughout the assessment. Participants were instructed to ensure the room being used could maintain testing conditions and privacy, without interruption for optimal conditions. Unforeseen environmental challenges were recorded and addressed on a case-by-case basis, as they would have been in for in-person administration.

4.7.3.2.4 Troubleshooting online administration

If connection issues arose, the participant would be automatically moved back to the private virtual waiting room, where the examiner could connect with them again. If

issues arose with the online administration platform, the examiner would call the participant and endeavour to troubleshoot the issues. If these could not be resolved, the assessment was postponed. Where the online assessment was not possible or practical, participants were invited to be followed up at a later time or for in-person assessment, Covid-19 social-distancing and travel guidelines permitting.

4.7.4 In-person administration

Some participants could not complete the study online or opted for an in-person appointment. This was performed in a quiet private room in the participant's home, Beaumont Hospital, or in the Biomedical Sciences Institute, TCD, depending on participant availability and preference. All Phase 1 MND Family study appointments were performed in-person. Most baseline prospective assessments for Phase 2 of the MND Family Study were carried out in-person, when Covid-19 restrictions permitted. This was due to the need for a blood sample and in-person administration of two neuropsychological tests (IGT and SART). Standard administration procedures were adhered. All stimuli were presented using a laptop (MacBook Pro 13") to maximise consistency between online and in-person administration. Questionnaires were completed on Qualtrics, in the same procedure as outlined above.

4.8 Prediction models of disease burden & manifestation in HD

There are a number of measures used to estimate hypothetical disease progression in PwHD or predict disease onset, leveraging the relationship between motor-symptom AoO and progression, and the number of CAG repeats on *HTT* (introduced in **section 1.6**).^{223,321} Three different predictive models/scores are used throughout the thesis for stratifying PwHD who have available CAG data (from clinical genetics). All models include age and CAG-repeat length. The calculation of time to predicted diagnosis⁷¹ in years, CAP100³¹⁵ and disease burden (**Equation 1**)³¹² are outlined in this section:

Equation 1: Penney et al.'s 'Disease Burden'

$$\text{'Disease Burden'} = \text{AGE} \times (\text{CAG} - L)$$

where, CAG is number of CAG repeats on *HTT* and *L* = is a constant representing lowest CAG at which pathology may arise = 35.5.³¹²

CAP100 is a standardised version of the CAG-Age-Product score (**Equation 2**).^{315,318}
This parameterisation anchors the score to 100 at the estimated age of motor onset.^{223,315}

Equation 2: CAP100

$$CAP100 = AGE \times \frac{(CAG - L)}{K}$$

where L, K = constants; when $L = 30$ and $K = 6.49$, expected age of motor-symptom onset will correspond to a CAP score of 100.^{223,315,318}

The most robust model using only CAG and age was created by Langbehn and colleagues^{71,314}, estimated time to predicted diagnosis (**Equation 3**). This model captures CAG-dependent variability in AoO the flexibility of which provides more accurate age-conditional risk predictions at different ages and CAG repeat lengths for premanifest individuals. Other models have similar predictive power, although overestimate risk at lower CAG lengths. This is particularly advantageous for the current study, where CAG-repeat lengths are relatively low.

Equation 3: Estimated Time to Diagnosis (years)

$$\left(1 + \exp \left\{ \frac{\pi}{\sqrt{3}} \frac{[-21.54 - \exp(9.56 - 0.146CAG) + Age]}{\sqrt{35.55 + \exp(17.72 - 0.327CAG)}} \right\} \right)^{-1}$$

Conditional survival probability equation for motor-symptom onset in premanifest HD based on age and CAG repeat number. From Langbehn et al. (2004).⁷¹

4.8.1 Stratifying premanifest PwHD

Based on this parametric survival mode and probable onset within 5 years, premanifest participants can be categorised as ‘far’ from (>10 years) and ‘near’ to estimated diagnosis (≤10 years). This is a prediction model, and due to variability in manifestation, it produces a ‘past’ (<0) group who are past their expected AoO, but have not developed motor-symptoms warranting clinical diagnosis.^{71,346,392}

4.9 Statistical considerations

Several statistical techniques were employed at the group level, including t-tests for two groups, analysis of variance for more than two, tests for categorical group differences (e.g.: Fisher exact test, Chi-squared tests), correlations, Intra-class correlations and regression-based analyses, multilevel linear level modelling, and other techniques to examine psychometric and reliability characteristics, described in their rel. An overview of the statistical methodologies shared across studies and that support the statistical analyses in this theses will be outlined here. Particular statistical techniques used will be elaborated upon in each relevant chapter. Analyses were performed using R (version 4.2.3) on RStudio (2023). The following R packages were used across all analyses: “arsenal”, “tidyverse”, “ggplot2”, “ggdist”, “pastecs”, “descr”, “effectsize”, “multcomp”, “reshape”, “WRS2”, “car”, “ez”, “lme4”, “lmerTest”, “performance”, “emmeans”, “rstatix”, “ggpubr”, “skimr”, “EnvStats”, “report”, “clubSandwich”, “TOSTER”, “knitr”.

4.9.1 Statistical power & sample size

For all tests, the alpha-level (α) was set at 0.05 with a desired power ($1 - \beta$) of 0.8, unless otherwise specified. All tests were two-sided, with the exception of the two one-sided t-test (TOST) presented in **Chapter 6**. Statistical significance therefore is considered $p < .05$. $\beta = 0.2$ implies there is a 20% chance of a Type II error (false negative or missed effect), and a true effect 80% of the time. For $\alpha = 0.05$, if the null hypothesis (H_0) is true, the probability of Type I error (a false positive) is 5%.

Sample size calculations were carried out to ensure sufficient statistical power at study design for the detection of meaningful effects, if they are present.⁵⁹⁶ A minimum sample size is set to reduce the possibility of Type II error whilst conserving resources where possible. Calculations were carried out to address three main comparisons of neuropsychological test scores: a) healthy controls and unaffected fALS relatives, b) healthy controls and manifest PwHD patients, and c) healthy controls and premanifest PwHD gene carriers.

Phonemic verbal fluency is the most robust marker of cognitive dysfunction in ALS in asymptomatic gene carriers⁴⁸⁹ and pwALS.¹⁶ Therefore, for the relatives of fALS patients, which are reported larger than relatives in general, expected effect sizes were based on this variable from Costello et al.^{194,210} and set as a large effect ($d=0.8$). Smaller effect sizes, with larger variation, are expected on neuropsychiatric measures for unaffected relatives ($d=0.5$). For HD studies, effect sizes were expected to be large between control

participants and early manifest PwHD ($d = 0.9$) and small to medium for premanifest HD participants ($d=0.5$) based on Paulsen and colleagues.³⁴⁶ Parameters for estimated effect sizes, desired power, and alpha level were entered into G*Power (version 3.1.9.6) to perform a priori sample size calculations.^{597,598}

4.9.1.1 *Unaffected fALS relatives prospective replication study*

To enable t-test comparisons between fALS relatives and healthy controls with an effect size of 0.8, desired power of 0.8 and alpha-level of .05, a sample size of 52 is needed, or 26 per group. For detection of differences for neuropsychiatric traits, the same parameters were applied, but with lower expected effect size ($d=0.5$). The minimum sample size in this case was calculated as $n = 128$, or 64 per group for the prospective MND Family replication Study.

4.9.1.2 *Power analyses for repeated-measures data*

G*Power does not natively support linear mixed-effects models (LMMs), which are used for analysis of the unbalanced longitudinal design with repeated measures. Therefore no formal power analysis could be performed. A common approach for analysis of sample size in longitudinal designs is to use repeated measures ANOVA as a proxy in this software package.⁵⁹⁹ For these calculations, estimated non-sphericity correction is set to, $\epsilon = 1$, because LMMs do not assume sphericity and therefore are robust to this assumption. Correlation among repeated measures of $r = 0.5$ was assumed to reflect a moderate level of within-subject stability across timepoints, consistent with literature on longitudinal cognitive outcomes.^{596,597}

Additional parameters for longitudinal follow up of the Phase 1 fALS relatives sample include an expected effect size of Cohen's $f = 0.3$ (equivalent to Cohen's $d = 0.6$)⁶⁰⁰, for two groups over two timepoints, with desired power of 0.8 and alpha-level of 0.05. G*Power recommended a minimum sample size of $n = 24$. For smaller effects in neuropsychiatric measures where expected effect size $f = 0.2$ (equivalent to $d = 0.4$), minimum required sample size is $n = 52$. As this was a retrospective follow-up of the prospective Phase 1 study, sample size for effects at follow-up are estimated, which do not account for attrition from baseline to T2.

The longitudinal aspect of the HD-Cognition study had three groups in the hypothesis testing (excluding Genotype Unknown participants). Planned design was for three timepoints, 12 to 18 months apart. Desired power and Type I error rate (α) were once again set to 0.8 and 0.05 respectively. To make sure effects for cognitive variables within the premanifest group were captured if present, the estimated effect size was set to $f =$

0.25 to allow investigation of interaction effects (group x time), and the inclusion of covariates. G*Power estimated minimal total sample size to be $n = 36$.

Drop-out rate must be accounted for over repeated-measure timepoints. A conservative estimate can be taken from studies in ALS, where high attrition is common due to rapidity of disease progression and disability. Using Elamin et al.¹⁷⁰ as the exemplar, the lowest rate of attrition was 50% over 1 year. HD has slower progression than ALS, and therefore attrition is likely to be lower, and more comparable to controls, which was approximately 40% in Elamin's study. Longitudinal analyses within the TRACK-HD study experienced participant dropout of approximately 10–15% per year across different sites.^{255,262} Therefore as a balanced estimate, attrition was expected at 40% over three timepoints (**Equation 8**). Total sample size for the HD-Cognition study was therefore $n'' = 60$ ($n=20$ per group).

Equation 4: Accounting for attrition in sample size calculation

$$n'' = \frac{n}{(1-q)}$$

where n = required sample size, and q = dropout rate = 0.4.⁶⁰¹

4.9.2 Group comparisons

Group mean scores are highlighted graphically with violin plots overlaid with box plots to illustrate distribution and measures of central tendency. Welch's t-test are used to compare two groups. It is a more robust and widely useable test than the Student's t-test, as it does not assume equal variances between groups. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) is applied. When necessary to control for the effects of additional variables (covariates), analysis of covariance (ANCOVA), were used. Post-hoc pairwise comparisons are conducted using estimated marginal means with Benjamini-Hochberg (BH) correction. Estimated marginal means are preferred as they incorporate model-derived parameters, including standard errors, degrees of freedom, and any covariates included in the model in determining significant group difference. This approach also appropriately accounts for unequal group sizes and non-constant variances by using model-estimated variances rather than assuming homogeneity of variance across groups. LMMs examine the longitudinal effects of group membership on neuropsychological outcomes over time and with complex data-structures (described below).

Study-specific control groups are used in each, unless otherwise stated. In the HD-Cognition study, primary comparisons are between three groups: individuals with manifest PwHD, premanifest PwHD and controls. In the MND Family Study, unaffected 1st and 2nd-degree relatives of individuals with fALS are compared to control participants without a family history of ALS. Phase 1 included sALS relatives as a third group, but current studies focus only on fALS relatives.

4.9.2.1 Planned sub-group comparisons

Depending on the *a priori* hypothesis, stated in the previous chapter, contrasts and sub-group analyses are performed using planned or post-hoc comparisons. Sub-group analyses differ depending on the study, and will be outlined more fully in subsequent chapters. As an overview, planned comparisons in the MND Family Study on cognitive endophenotypes and asymptomatic cognitive function compares *C9orf72*-positive and -negative unaffected relatives of pwALS over time.

In HD-Cognition, premanifest HD participants, categorised by proximity to predicted clinical diagnosis (“near” vs. “far” and “past”) are compared to each other and controls. In **Chapter 5**, pwALS are grouped according to endorsed distress levels, comparing ‘high’ and ‘low’ distress groups on neuropsychological measures. In all cases, as outlined above, correction for multiple comparison will be performed as appropriate.

4.9.3 Linear mixed effects models

Multi-level linear models, or Linear mixed-effects models (LMMs) are a powerful statistical technique for modelling individual-level variability, accounting for data dependence, often overlooked in traditional methods such as t-tests and ANOVA.⁶⁰² This is particularly important for repeated-measures designs and when participants are nested within various levels, such as individuals within a family—as they are in the MND Family study. Statistical power is preserved in LMMs due to the more accurate estimation of error terms and greater flexibility with missing data, reducing the loss of degrees of freedom necessitated by traditional methods (i.e.: repeated-measures ANOVA). When data are missing at random for a given time point, multilevel models can accommodate the missing data by estimating parameters using the available data, allowing the case to remain in the analysis rather than being excluded entirely. They also model variance-covariance structures directly, and therefore do not require the sphericity assumption to be met.^{602,603}

LMMs are used to examine longitudinal effects of group membership on neuropsychological outcomes over time in the current studies. The R packages ‘lme4’,

‘lme4’ and ‘lmerTest’⁶⁰⁴ are used to fit models over two levels of time (Level 1) and group (Level 2). Initially fitted with Maximum Likelihood (ML) estimation to compare model fit, selection was guided using likelihood ratio tests (LRTs) and ANOVAs to compare models. Selected models were those which showed significant improvements (decreases) in log-likelihood and Akaike information criterion (AIC) values from baseline or more complex models. When the best fitting model was selected, it was refitted using Restricted Maximum Likelihood (REML) estimation to obtain more unbiased estimates of the variance components (random effects). Satterthwaite approximations were used to derive p-values and degrees of freedom, which offer Type I error control in small samples.⁶⁰⁵

Meteyard and Davies (2020)⁶⁰⁶ review best practice guidelines for the use of LMMs in psychological research, which are largely followed here. Model diagnostics are assessed as below (section 4.9.5), ensuring model assumptions are met. In cases where residual diagnostics indicate violations of model assumptions (e.g.: heteroscedasticity, non-normality) unaddressed by other methods, cluster-robust standard errors were computed using the ‘clubSandwich’ package in R.⁶⁰⁷ This method implements cluster-robust variance estimation (CR2), which adjusts standard errors and p-values for dependency within repeated measures using Satterthwaite degrees of freedom, and is specifically suited for use with LMMs.^{607,608}

4.9.4 Other regression-based considerations

Bivariate associations were examined using Pearson’s correlation coefficient (r), with effect size (r), direction, and significance reported. In cases of non-normality or small sample sizes ($n < 30$), Spearman’s rank correlation (ρ) was used. Linear regression models are used to examine the effects of continuous predictors (e.g.: disease burden, age) on neuropsychological test scores. Hierarchical regression assessed the extent to which group differences on test scores were accounted for by potentially confounding or moderating variables. Covariates and interaction terms are considered for inclusion on a case-by-case basis. Shared variance (R^2) was reported, where informative.

Logistic and binary logistic regression were applied for categorical and dichotomous outcome variables respectively. Analyses met the minimum expected frequency assumptions for contingency tables, and were discontinued in cases of complete separation, where predictors perfectly predicted the outcome. Other common assumptions are described below (4.9.5).

In ANOVA and simple/multiple linear regression models when the assumption of homoscedasticity was violated (**4.9.5**), robust heteroscedasticity-consistent (HC3) standard errors were applied using the ‘sandwich’ (v3.0-2) package in R. Adjusted model coefficients and statistical significance were derived from this using the ‘lmtest’ (v0.9-40) package in R, reported where necessary to account for non-constant variance in residuals.

4.9.5 Assumptions for statistical tests & addressing violations

Statistical assumptions relevant to each analysis type were assessed systematically. For linear regression, general linear models and LMMs, assumptions of linearity, homoscedasticity, normality of residuals, and independence were checked statistically and with visual inspection using the ‘performance’ package in R. Linearity and homoscedasticity were evaluated using scatterplots of residuals against fitted values. A random scatter of points around zero was expected; systematic patterns indicated violations. Normality of residuals was assessed via Q-Q plots and Shapiro-Wilk tests. Multicollinearity was assessed using the variance inflation factor (VIF), with predictors showing $VIF > 5$ considered for exclusion. High-leverage/influential observations were identified using Cook’s distance (threshold >1). Sensitivity analyses were conducted by refitting the model without high leverage cases to assess the stability of model estimates. If the exclusion resulted in substantial changes to parameter estimates or model fit, their influence was considered further. However, if model results remained stable, high-leverage cases were retained in the final model and reported.

4.9.5.1 Assumption of normality

A p-value $<.05$ on a Shapiro-Wilk test suggested deviation from normality. These test scores are often over-inflated. Sampling distributions with relatively light tails will be normal if the sample size is large enough (typically $N > 30$).⁶⁰⁹ For example, in the ECAS, used in all studies to follow, scoring is on a fixed scale (0-136) with count-like properties. The distribution of scores are inherently skewed, which limits standard parametric techniques in interpreting scores.¹⁶⁹ However, the central limit theorem (CLT) indicates that the sampling distribution of the data/regression residuals will approximate a normal distribution, regardless of the underlying distribution. This occurs because, as sample size increases, the standard error of the estimate decreases (i.e.: standard deviation divided by the square root of N), stabilising the estimate and reducing the influence of non-normality. As a result, violations of normality assumptions for predictors or residuals tend to have minimal impact on the validity of regression estimates in large samples.^{603,610}

4.9.5.2 Other assumptions

For mixed-effects models, residual diagnostics were similarly conducted. Additionally, the distribution of random effects was inspected for violations of normality. Homogeneity of the regression slopes is an ANCOVA-specific assumption. This requires that the relationship between the covariate and the outcome variable are consistent across all levels of group. It is checked by modelling the interaction term. A significant interaction would suggest differential effects of the covariate across groups, leading to biased conclusions, therefore violating the assumption.

In instances where assumptions are violated, alternative strategies were considered, including Welch's F-test (does not assume equal variances), transformations and robust regression techniques. Kruskal-Wallis (>3 groups) or Mann-Whitney U/Wilcoxon rank-sum test for independent samples (2 groups) and Wilcoxon signed-rank test for paired data tests (2 timepoints) offered alternatives for non-parametric data. To determine group effects within a Kruskal-Wallis test, post-hoc Wilcoxon rank-sum test were performed with Benjamini-Hochberg correction for multiple comparisons. Assumption violations and the actions taken to address them were explicitly reported in relevant result sections.

4.9.6 Correction for multiple comparisons

Participants were compared on a number of different neuropsychological measures across domains. Multiple comparisons increase the probability of Type I errors. The Bonferroni and Šidák corrections are traditional methods for controlling Type I error rates, but their conservatism increases as the number of comparisons and degree of correlation between outcome measures rises, leading to reduced statistical power.⁶¹¹ Therefore, when necessary to control for multiple comparisons, the Benjamini-Hochberg method is used.^{612,613} This stepped method corrects for the False Discovery Rate (FDR) and Type I error rate inflation by estimating the proportion of H_0 which have been falsely rejected (**Equation 5**).⁶⁰³ p-values are ranked from smallest to largest, and significance is determined based on a calculated critical value for each using the 'stats' package in R. This ensures that the chance of falsely rejecting the null hypothesis remains controlled across the set of significant findings. FDR is less conservative and thus more powerful than Bonferroni corrections. It controls the proportion of false positives among significant results rather than the overall familywise error rate, similar to the Holm correction.⁶¹⁴ The FDR approach is especially suitable in neuropsychological research where numerous

correlated outcomes (e.g.: cognitive domains or behavioural scores) are examined, and a balance between sensitivity and specificity is required. p-values are. Statistical significance after correction is denoted by “p.adj” <.05.

Equation 5: *Correction for multiple comparisons*

$$\text{a)} \quad FDR = \frac{\text{number of falsely rejected } H_0}{\text{total number of rejected } H_0}$$

$$\text{b)} \quad p_{crit} = \frac{j}{k} \alpha$$

a) False Discovery Rate, b) critical p-value for the FDR method, where j = p-value rank, k = total number of comparisons, and α = chosen error rate = 0.05.

4.9.7 Other measures of statistical inference

4.9.7.1 Test-retest & inter-rater reliability

In this thesis, ICCs were employed across three contexts: to evaluate inter-rater agreement in scoring neuropsychological assessments for new assessors, to assess the strength of clustering within nested levels of multilevel data and to determine test-retest reliability in the validation of the online version of the neuropsychological battery (**Chapter 5**). ICC is a preferred statistic in these contexts as it can reflect both the consistency and absolute agreement between scores, depending on the model selected. The specific form of ICC applied varies depending on the design of each analysis. Full details, including model specifications, type, and the definition of ICCs used, are reported in the relevant study results chapters, in line with established guidelines for reliability research.⁶¹⁵ The general structure is as follows:

4.9.7.2 Test validity

To evaluate the diagnostic utility and validity of the ECAS in HD (**Chapter 10**), against gold-standard neuropsychological measures sensitivity, specificity & predictive values were calculated (**Equation 6**). In addition, predictive values were computed to assess the clinical utility of the measures.⁶¹⁶ Positive Predictive Value (PPV) indicated the proportion of true positives among all positive test results, and Negative Predictive Value (NPV) reflected the proportion of true negatives among all negative test results. High levels

of each are desirable, with sensitivity and NPV particularly important for screening measures.

Equation 6: Measures of test validity

$$\begin{aligned} \text{Sensitivity} &= \frac{TP}{TP + FN}; & \text{PPV} &= \frac{TP}{TP + FP}; \\ \text{Specificity} &= \frac{TN}{TN + FP}; & \text{NPV} &= \frac{TN}{TN + FN}. \end{aligned}$$

where, TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative, PPV = Positive Predictive Value, NPV = Negative Predictive Value.

4.9.7.2.1 Abnormality classification

Test-specific published cut-offs were used to categorise abnormality/impairment, if appropriate. For tasks without published cut-offs, the cut-off for impairment was set at $z = -1.65$ from the control mean, or the 5th percentile. Determinants of decline (Reliable Change Indices) are also used to detect change over time, where relevant.^{162,163,536}

4.9.7.2.2 ROC curve analyses

Receiver operating characteristic (ROC) curve analyses are performed to evaluate the diagnostic accuracy of a test by plotting sensitivity against the false positive rate (1 – specificity) across a range of threshold values. This determines a test’s discriminative ability and optimal cut-off points. Binary group classification (e.g.: gold-standard vs screening test) serve as the dependent variable. ROC curves are generated using the ‘pROC’ package in R.⁶¹⁷ The area under the curve (AUC) represents the probability that the test correctly distinguishes two groups. Higher AUCs denoting better overall test performance. Optimal classification thresholds were determined using Youden’s J statistic, which maximises the balance between sensitivity and specificity (sensitivity + specificity – 1), avoiding false positives. Pairwise comparisons of AUCs were conducted using DeLong’s test for correlated ROC curves.⁶¹⁸

4.9.7.3 Confidence intervals

Confidence intervals (CI) quantify the uncertainty around statistical estimates. Unlike p-values, which indicate the probability of observing data under the null hypothesis (H_0), confidence intervals provide a range within which the true population parameter is

likely to lie. A 95% confidence interval (95% CI) means that if multiple samples were taken from the same population with a 95% confidence interval computed for each, the true population mean would be expected to fall within 95% of those intervals. Wider intervals reflect greater uncertainty, often due to small sample sizes, whereas narrower intervals indicate more precise estimates, typically seen in larger samples with higher power. Tighter confidence intervals offer greater confidence in the stability of the estimates and also allows comparison of the magnitude of a difference.⁶¹⁹

4.9.7.4 Measures of effect size

Effect sizes are another quantitative estimate of the strength or magnitude of an observed effect. While p-values indicate whether an effect is likely to have occurred by chance, effect sizes are standardised measures that help determine the practical relevance of that effect appropriate to the specific statistical context.⁶⁰³ Cohen's d, Pearson's r, partial eta-squared (η_p^2) and omega squared (ω^2) effect sizes are reported throughout this thesis, selected according to the type of analysis conducted.^{600,620} Effect sizes are generally interpreted following Cohen's conventions.⁶²⁰ For example, Cohen's d: small = 0.20, medium = 0.50, large = 0.80. Cohen also provides interpretation for alternative effect-size statistics, including η_p^2 , interpreted as: small = 0.01, moderate = 0.06, and large = 0.14, and r, interpreted as: small = 0.10, moderate = 0.30, and large = 0.50. For LMMs, values >0.3 were interpreted as large for marginal R² (fixed effects), and >0.6 as large for conditional R² (total variance explained).⁶²¹

Effect sizes for non-parametric Kruskal-Wallis tests are estimated using the ϵ^2 statistic, calculated using the 'kruskal_effsize' package in R (**Equation 7**).⁶²² It is similar to the η^2 statistic, interpreted on a similar scale, calculated as follows:

Equation 7: Epsilon-squared (ϵ^2) for estimating Kruskal-Wallis effect size.

$$\epsilon^2 = \frac{H - k + 1}{n - k}$$

Where, H = Kruskal-Wallis test statistic, k = number of groups, n = total number of observations.

4.9.8 Handling missing data

When using models robust to missingness, such as LMMs, and data were presumed to be missing at random, incomplete data points were retained in the analysis.

These models use maximum likelihood estimation, which allows for inclusion of cases with partially missing data under the assumption of missing at random.^{603,623} This avoids unnecessary case-wise deletion and preserves statistical power. For analyses that required complete data (e.g.: certain summary score derivations), listwise deletion was applied. Where domain/scale scores were dependent on fully completed questionnaires, forced-response formats were implemented in Qualtrics to reduce missing data at source. Imputation was not performed.

4.9.9 Differential deficits & the Relative Fluency Index

Phonemic verbal fluency is differentially affected in ALSci, with the relative sparing of semantic fluency performance.¹¹⁸ Semantic fluency is an easier task, predominantly of temporal lobe function.¹⁴⁵ A differential deficit-type discrepancy score, contrasting semantic with phonemic verbal fluency performance, therefore may provide more sensitivity to this cognitive biomarker for individual pwALS, and at the group level.⁶²⁴ An ALS-specific differential cognitive score is proposed.

To express the Relative Fluency Index (RFI) score, the difference between semantic and phonemic fluency z-scores is divided by standard deviation of the difference between the z-scored values.⁶²⁴ z-scores should be standardised from age- and education-stratified normative data.⁵⁶¹ This yields a standardised difference score with a standard deviation of 1 under assumption of independence where controls are expected to perform at $z = 0$ (the mean). Created based on the Revised Standardised Difference Test⁵²⁶, it is as follows:

Equation 8: *Relative Fluency Index (RFI) gives a standardised difference score between the two z-scored fluency measures.*

$$\text{Relative Fluency Index (RFI)} = \frac{Z_{\text{phonemic}} - Z_{\text{semantic}}}{\sqrt{2 - 2 * r_{xy}}}$$

Where:

- $Z_{\text{phonemic}}, Z_{\text{semantic}}$: z-transformed FAS and semantic fluency scores;
- r_{xy} : correlation between the two tests in control sample.

Semantic and phonemic fluency are strongly correlated, and so it is expected that r_{xy} will be between 0.4 and 0.6.^{118,146,625} Overall, larger negative scores on the RFI indicate a larger discrepancy and worse *relative* performance on phonemic verbal fluency.

4.10 Ethical considerations & data protection

Ethical approval was sought and approved for all studies, including amendments to procedures necessitated by the Covid-19 pandemic. Studies were carried out in accordance of the declaration of Helsinki and were approved by the Beaumont Hospital Research Ethics Committee (reference: MND Family study (pwALS and relatives): #15/40; HD-Cognition: #18/45).

All patients provided fully informed consent. Study information leaflets and consent forms were prepared in clear English. Individuals were made fully aware of the study rationale, procedures, risks, benefits, confidentiality and data protection measures. The consent process included review of study information leaflets with time allowed for consideration and questions before written consent was provided. This was to ensure due consideration was given to participation by the participant, that they did not feel pressure to participate, and to give time for any concerns to be addressed by the researchers. Participants were assured that they could decline or withdraw participation at any time, and that their clinical care, and/or that of their family member would not be affected.

There were no immediate benefits to the participants by partaking in the outlined studies, nor were there any potential harms. Participants may experience, a) fatigue as a result of taking part in the long neuropsychological assessments, b) unpleasant feelings (anxiety, low mood) arising due to engagement in research on conditions which affect them/their family or for which they may be at genetic risk for, and c) discomfort and bruising due to phlebotomy, for participants who provide blood sample for genetic testing. To mitigate these, participants were encouraged to take breaks if needed, and could discontinue the assessment at any time. Researchers had training in open disclosure and were clinical-facing with experience with these populations.

Verbal consent was sought at the end of each assessment in a longitudinal design to contact the participant to arrange the subsequent appointment. Verbal consent was again obtained prior to each follow-up appointment in longitudinal studies. All participants who agreed to longitudinal follow-up post Covid-19 amendments were re-consented to the studies under the new procedures and amended ethics documents.

4.10.1 *Data processing & storage*

All participants were assigned a unique participant study ID. In the MND Family study, this identified family and the individual within the family. Neuropsychological assessment data were scored according to published manual instructions on hard copy

record forms. Data were transferred to excel spreadsheet databases, where they were codified, identifiable only by their unique participant ID. In accordance with particular study ethical approval requirements, databases were password protected and stored on secure machines and/or servers. Hard copy data were stored in locked filing-cabinets. A separate password protected recruitment codebook stored the unique participant study IDs with identifiable information necessary for recruitment and GDPR compliance. Clinical, genetic and research data were not stored with identifiable information.

Data protection impact assessments (DPIA) were performed, with approval sought from Beaumont Hospital data protection officer(s). Participants were informed that they could withdraw their data from the study at any time. All obtained data were used for research purposes only, and will be stored and disposed of according to the GDPR regulation and study-specific requirements, as approved by Beaumont Hospital Research Ethics Committee.

4.10.2 *Safety & risk management*

A risk assessment was carried out by the PhD student (CP) with the Principal Clinical Neuropsychologist for the online assessment of patients. A risk protocol subsequently created (**Appendix 2**). At the start of every online assessment, the limits of confidentiality and the principles of fully informed consent were discussed, and any questions pertaining to risk were addressed. Participants were informed of the resources available to them, should extra support be required. Confidentiality and risk-mitigation information were provided to participants before and after all relevant online questionnaires respectively (**Appendix 4**).

5 Chapter 5:

Results: The testing environment

5.1 Chapter overview

This chapter presents two studies examining how contextual factors influence neuropsychological test performance. The first validates an online assessment battery developed during the Covid-19 pandemic, and the second investigates how clinic-based variables—including distress, tiredness, and proximity to diagnosis—affect cognitive screening outcomes in pwALS.

5.2 Introduction 1

5.2.1 The effects of the Covid-19 pandemic: Online battery validation & feasibility

The work associated with this PhD research began in November 2020, and was for the most part, a continuation of studies which had already begun prior to the Covid-19 pandemic. To allow the studies continue during this time, original protocols had to change. Online administration of the neuropsychological battery was deemed to be the most viable solution long term in the context of changing guidelines around travel, social distancing and safety, as well as to protect the health of the participants, many of whom were at high risk of more severe disease and associated complications from Covid-19. The neuropsychological assessment battery was therefore adapted for use online via video call. Although the test stimuli and administration instructions were largely the same for online administration, the medium may have affected test validity. It was important for continuity and consistency over time that the online versions were adequately validated against the standard in-person administration, and so a small validation study was performed, making up the first part of this chapter.

5.2.2 *Tele-neuropsychology*

Online administration of cognitive and neuropsychological assessments has become more commonplace since the Covid-19 pandemic. The context validity and general utility of these methods is reinforced by studies showing negligible differences between online and in-person assessment, considering certain conditions of quality are met. Marra and colleagues⁶²⁶ carried out a systematic review of counterbalanced crossover studies investigating differences between standard face-to-face administration of neuropsychological measures and remote or online versions, building on similar systematic review and meta-analytic work by Brearly in 2017.⁵⁹³ They provide test-level validity for specific measures and strong support for the use of many of the tests in this battery via the online medium. Verbally-mediated tasks, such as digit span, verbal fluency and list learning tasks in particular were unaffected by videoconference administration, and visually-dependent tasks also showed consistency between test-environments.^{593,627} Whilst they acknowledge many tests do not have sufficient evidence, particularly tasks of executive function and processing speed, almost all measures addressed offer promising reliability between standard face-to-face and remote administration.^{626,628}

For questionnaire data, mean scores and internal consistency of online versions of questionnaires have not been shown to differ significantly from pen and paper-based versions^{589,629}, including the BBI specifically.⁶³⁰ Therefore, it was assumed that using Qualtrics to collect these responses would not alter participants' scores or the validity of the measures, once questionnaire structure and instructions remained unchanged in transcription. Using Qualtrics may afford benefits to pen and paper questionnaires, by reducing missing data and improving the completeness of the data collected via the 'forced response' feature.

5.2.3 *Chapter Aims*

This study addresses **Aim 1**, to adapt and validate an online version of the neuropsychological assessment battery. It is hypothesised that the online assessment battery will show acceptable reliability and validity against the standard in-person administration.

5.3 Methods 1

5.3.1 Sample size calculation

Calculations for minimum sample size, k , in a repeated measures test-retest-reliability design were carried out to ensure the study was adequately powered. Desired power was set at 0.8 ($\beta=0.2$), with an alpha level of $\alpha=0.05$. For the assessment of reliability, considering minimum acceptable reliability of $\rho_0=0.6$ and expected reliability of $\rho_1=0.9$ were used.⁶³¹ Two timepoints, or replicates ($n=2$), are performed, in-person and online. Between- and within-subject variation are considered as components of reliability in this method. A minimum sample size of $k=11.7$, was deemed adequate.

5.3.2 Participants

12 healthy control participants who had taken part in previous neuropsychological research in the Academic Unit of Neurology were recruited, reported in Pinto-Grau et al. (2021).⁵³² They met the inclusion/exclusion criteria for healthy controls outlined in **Chapter 5**. The sample demographic characteristics are provided in **Table 8**. The same administration procedures and instructions were used, as per published guidelines or test manuals, with the exception of the online medium.

Participants were aged between 43 and 59 at baseline assessment, 42% of whom were female ($n=5/12$). They completed their baseline assessment between March 2016 and April 2018 in the initial study. T2 assessments were completed online in early 2021, for an interval of 3.75 to 6.25 years between time points. Examiners differed, but they had previously undergone inter-rater reliability procedures to ensure administration and scoring of the tests were consistent.

5.3.3 Procedure

The validation sample completed a standard in-person assessment battery as part of a larger study on neuropsychological function in ALS. They were contacted and reconsented to the current study, as per study procedures. Appointments were performed using videoconferencing software. For tests requiring the presentation of stimuli, the examiner's screen was shared, displaying the stimulus to the participant. All other procedures are followed as per the general study procedures.

A specifications protocol for participant environment and technology was created to ensure private and quiet testing conditions, as would be provided in a clinical facility, and minimum device screen size specifications were set to ensure stimuli met an acceptable threshold (tablet or laptop screen larger than 9") based on the tele-neuropsychology literature.⁵⁹³ Minimum requirements for Wi-Fi efficiency also had to be met, with picture and sound clarity the measure of this (i.e.: no time lag between presented stimuli or spoken words). If these conditions were not met, the assessment was terminated. Online safety and risk protocols were created to safeguard participants and researchers (**Appendix 4**).

5.3.4 Materials

Each participant completed the online 'MND Family Study' neuropsychological test battery. Tests included for each study battery are shown in **Table 6**. The ECAS is an integral part of each study. Participants did not complete the ECAS at their first timepoint. However, a remote version of the ECAS, suitable for online administration, was created by the original authors.^{159,595} This was made available to researchers by request from <https://ecas.psy.ed.ac.uk/remote-ecas/>. The authors completed a reliability, validity and acceptability study of the remote version, the findings of which have since been published showing equivalent scores and high agreement between the administration methods.⁵⁹⁵ They also described positive feedback on the feasibility of the remote assessment in pwALS.

The ACS Social Perception Battery could not be validated directly as it was a new addition to the test battery. Administration guidelines were adhered to, and stimuli consisted of similar methods of administration as other tests, which were validated. The Symbol Digit Modalities test (SDMT) was not part of the original battery and so could not be included in the validation. However, normative data are available for orally administered versions which, were used for the online administration.^{553,554}

5.3.5 Statistical considerations

Reliability in this sense, is the degree of consistency in the interpretation of a test or instrument across different situations, x and y .⁶⁰³ In this case, y = in-person/baseline scores, x = online/follow-up scores and c = systematic error:

$$y = x + c$$

Equation 9: Reliability is the degree to which a score in one situation (y) can be equated to a score in another (x), whilst factoring in systematic error (c).⁶¹⁵

An two-way mixed-effects ICC model with mean-rating for consistency was used to assess test-retest reliability.⁶¹⁵ Two-way mixed-effects method was selected as there were only two examiners in the study, and the sample cannot be considered as randomised due to the repeated measures design.

The null hypothesis is $H_0: \rho = \rho_0$, where ρ_0 is a specified value of ρ (which in this case is 0.6) and the alternative hypothesis is $H_1: \rho > \rho_0$. This method is most suitable for this evaluation as it assesses the relative consistency and ranking of scores, ignoring any systematic differences in the absolute scores across the testing environments. The equation for this method is:

$$ICC(3, k) = \rho = \frac{MS_R - MS_E}{MS_R}$$

Equation 10: Two-way ICC for average scores for consistency. k = number of measurements; ρ = Intra-Class Correlation coefficient; MS_R = Mean Square of the rows; MS_E = Mean Square of the error.⁶³²

Practice effects were also considered as the mean performance increases across the two administrations. Paired sample t-tests with Benjamini-Hochberg correction False Discovery Rate (FDR) formally tested the practice effects between the two sessions, correcting for multiple comparisons.

5.4 Results 1: Online validation study

The average duration between the baseline, in-person assessment and the online assessment for these participants was 4.69 years. The ICC analysis found that the majority of tests were above the minimum reliability threshold of $\rho_0=0.6$, showing good to excellent reliability (**Table 8**).⁶¹⁵

Two tests were not statistically significant at the minimum reliability threshold. These included post-interference recall on the RAVLT ($\rho=0.636$, $p=.054$) and the recognition subtest of the RCFT ($\rho=0.416$, $p=.19$). Examining these task mean scores more closely, the RCFT recognition had a mean(standard deviation) of 20.67(1.3) at T1 and 20.5(1.57) at T2, and the RAVLT post-interference recall subtest had means of 11.25(2.93) at T1 and 11.42(3.99) at T2. The upper limits of the confidence intervals on these tests are also well above the minimum reliability threshold at 0.83 and 0.9 respectively. Both of these tasks are likely affected by a lack of variance in the data, which could produce lower ICC values despite minimal variability in the mean scores and good to excellent upper limits of the confidence intervals. Furthermore, FDR-corrected paired one-sample t-tests showed no significant differences in performance on any test indicating, learning effects and differences between face-to-face and online administrations were absent (**Table 8**).

The measure of current IQ was different between testing periods (Raven's Progressive Matrices vs WASI-II) and so could not be included here. However, the Raven's Progressive Matrices and the Matrix Reasoning subtest of the WASI-II assess the same domain of fluid intelligence, with near identical administration methods. Scores did not differ on these tests ($t(11)=-0.507$, $p=.624$).

5.4.1 Feasibility & acceptability of the online assessment

The overall the acceptability of remote assessment was high. Participants cited convenience (e.g.: not having to travel) and comfort being at home as particular benefits of the online testing format. One participant stated that in-person testing would be preferable, but was happy to have the online alternative. Some internet connection issues interrupted a minority of sessions, and only infrequently. Although this did not interfere with presentation of stimuli or scoring, one participant reported mild frustration. One other potential participant did not partake in the validation study due to not having the appropriate technology (a computer or laptop with screen >9"), though this was rare for the controls contacted. In general, the participants reported no issues with using the online technology.

Table 8: Online validation study table of results

			Test-Retest Reliability				Practice Effects		
			ICC	F-Test <i>df</i> =(11,11)			t-test ^a <i>df</i> =11		
	T1 In-person M (SD)	T2 Online M (SD)		95% CI	F	<i>p</i>	<i>t</i>	<i>p</i> ^b	
n	12	12	-	-	-	-	-	-	
Sex (% Female)	41.7%	-	-	-	-	-	-	-	
Years Education	16.83 (2.62)	-	-	-	-	-	-	-	
Average testing interval (years)	-	4.69 (0.94)	-	-	-	-	-	-	
Age	52.83 (5.13)	57.67 (5.25)	-	-	-	-	-	-	
ECAS Total Status (% Normal)	-	100	-	-	-	-	-	-	
Raven’s progressive matrices FSIQ	111.99(3.78)	-	-	-	-	-	-	-	
WASI-II FSIQ	-	113.92(12.87)	-	-	-	-	-	-	
Premorbid IQ (TOPF FSIQ)	111.92 (11.29)	112.8 (8.55)	0.896	[.64,.97]	9.65	< .001	-0.49	0.919	
Reading the Mind in the Eyes Test (RMET)	27.636 (3.325)	26.833 (3.904)	0.837	[.43,.95]	6.12	.003	0.97	0.711	
RAVLT	Total 1-5	57.583 (9.793)	55.333 (11.633)	0.883	[.59,.97]	0.852	< .001	1.12	0.694
	Post-interference	11.250 (2.927)	11.417 (3.988)	0.636	[-0.26,.9]	2.75	.054*	-0.16	0.952
	Delayed recall	11.167 (3.326)	11.250 (3.980)	0.952	[.83,.99]	21	< .001	-0.18	0.952
	Recognition	47.583 (3.232)	46.750 (4.575)	0.746	[.12,.93]	3.94	.016	0.81	0.788
	Immediate recall	32.083 (5.468)	31.667 (7.203)	0.851	[.48,.96]	6.72	.002	0.31	0.952
Logical Memory	Delayed recall	29.083 (7.012)	27.667 (8.585)	0.875	[0.57,.97]	7.98	< .001	0.94	0.711
	Recognition	26.583 (2.065)	25.833 (2.250)	0.693	[-.07,.91]	3.21	.031	1.24	0.694
Rey Complex Figure Task (RCFT)	Copy Score	34.292 (2.988)	33.955 (3.004)	0.878	[.58,.97]	0.823	< .001	0.3	0.952
	Immediate Recall	23.208 (4.769)	25.318 (5.627)	0.891	[.62,.97]	9.19	< .001	-2.45	0.319
	Delayed Recall	23.000 (4.118)	24.364 (5.767)	0.816	[.36,.95]	5.44	.005	-1.21	0.694
	Recognition	20.667 (1.303)	20.500 (1.567)	0.416*	[-1.03,.83]	1.71	.193*	0.33	0.952
D-KEFS Colour Word Interference Test (CWIT)	Naming Time	29.667 (2.498)	28.583 (4.719)	0.78	[.24,.93]	4.55	.009	1.17	0.694
	Reading Time	23.917 (4.963)	21.167 (2.125)	0.699	[-.045,.91]	3.32	.029	2.59	0.319
	Inhibition Time	54.909 (9.904)	52.750 (11.779)	0.911	[.69,.97]	11.2	< .001	1.13	0.694
	Switching Time	62.27 (18.023)	63.167 (22.336)	0.778	[.23,.94]	4.5	.01	-0.19	0.952
Digit Span	Forward	11.500 (4.296)	12.083 (1.832)	0.88	[.58,.97]	8.33	< .001	1.47	0.694

		T1 In-person M (SD)	T2 Online M (SD)	Test-Retest Reliability				Practice Effects	
				ICC	F-Test df=(11,11)			t-test ^a df=11	
					95% CI	F	p	t	p ^b
Verbal Fluency	Backwards	9.583 (3.704)	10.917 (2.109)	0.766	[.19,.93]	4.27	.012	-0.62	0.888
	FAS Total	41.58 (11.603)	48.167 (11.731)	0.865	[.53,.96]	7.42	.001	-2.84	0.319
	Restricted (C) Total	7.500 (3.503)	8.250 (4.159)	0.735	[.08,.92]	3.77	.019	-0.74	0.812
	Semantic (Animals)	21.333 (5.140)	24.083 (6.842)	0.722	[.04,.92]	3.6	.022	-1.69	0.575
Boston Naming Test	Spontaneous	28.083 (1.782)	28.083 (1.621)	0.933	[.77,.98]	15	<.001	0	1
Action Naming Test	Spontaneous	27.500 (1.168)	27.500 (0.522)	0.75	[.13,.93]	4	.015	0	1

*non-significant test trials; Mean (Standard Deviation). ICC=Intra-Class Correlation; df=Degrees of Freedom; TOPF=Test of Premorbid Function-UK; FSIQ=Full Scale IQ estimate. a=paired one-sample t-test; b=Benjamini-Hochberg (FDR) correction applied.

5.5 Discussion 1

This validation study has shown that the majority of the tests used in the neuropsychological battery meet the minimum acceptability criteria demonstrating consistency across the in-person and online media, concordant with prior findings.^{593,626-628} Mean differences between testing sessions were negligible on these tests, indicating a) the online medium was not significantly easier nor more difficult than the in-person medium, and b) participants performance did not significantly improve because of practice effects. The time interval was large however (average 4.69 years), and so significant practice-effects are not expected.

There were non-significant improvements in performance on the timed tasks (verbal fluency, simple reading speed). Considering it is unlikely that learning effects may have impacted T2 scores, it could be that under timed conditions where performance anxiety or pressure might be higher when face-to-face with an examiner is lower when online, in ones' own home, without an examiner physically present.⁶³³ These factors are unlikely to significantly affect performance, but may contribute towards systematic variability and bias. This idea may be further supported by the acceptability findings, where participants often cited higher levels of comfort and ease of taking part online in their own homes. Acceptability and feasibility outcomes fed back into the creation of online protocols.

5.5.1 *Study specific limitations*

Connectivity issues during tasks with timed responses (e.g.: SDMT, CWIT), or where the stimuli cannot be repeated verbally (e.g.: RAVLT, Digit Span) could affect the standard administration. In the event of significant connectivity issues, this would be noted, and case-by-case decisions made about whether the validity of the test results were affected. If results were affected, the test scores were excluded. Albeit rarer, this could happen at in-person assessments due to noise or other interruptions in clinical settings or in a participant's home, and such decision making would also have to be made considering all factors. Most tests could be adapted for online administration, although some could not (IGT, SART). Alternative methods and software were evaluated for suitability of doing these tests remotely, but were not deemed adequate or possible, and so the tests were excluded from the assessment battery.

Follow up periods were long (mean=4.69 years), which may have affected scores. However, healthy controls in this age range are not expected to exhibit decline. Age-stratified normative data was used to standardise performance, which would mitigate age-related variability. Additionally, this interval is similar to that in the longitudinal arm of the MND Family study, discussed in **Chapter 6**.

5.5.1.1 Considerations of online administration

This validation sample were healthy control participants. It is important to also consider the feasibility of the online assessment for participants with neurological conditions, as well as their acceptability. Motor disability or visual impairment can impact feasibility, but such limitations are not significant in other studies of remote assessment in ALS.⁶³⁴

Using the oral version of the SDMT may affect the generalisability of the SDMT findings, especially in comparing the different versions, and as the written version is standard. However, the scores were converted to z-scores using the normative data stratified for age and education, comparisons between scores are comparable to each other as relative measures. One benefit of the oral version of the SDMT over the written administration is that it minimises the confounding effects of hand motor function.

5.6 Conclusion 1

Adaptation of the neuropsychological assessment battery for use online has shown good reliability and high acceptability by this sample to allow continuation of these studies. Considerations may need to be made for participants with neurological disability where fatigue and motor or visual impairment may affect task engagement and external validity, which are considered on a case-by-case basis, as they would be in standard in-person administration. Challenges with the online administration informed protocol development to ensure generalisability and validity of future administration and data collection.

5.7 Chapter 5, part 2: Cognition in the clinic environment – distress & factors affecting neuropsychological screening tool outcomes

5.8 Introduction 2: Screening for cognitive impairment

The ECAS is the gold-standard screening assessment for detection of cognitive change in ALS, created in line with the Strong Criteria for characterising ALS with cognitive and/or behavioural impairment, ALSci & ALSbi respectively.^{16,159} Although it remains unclear whether cognition declines with disease progression¹¹⁵, best practice guidelines recommend screening for cognitive impairment at a patient's first visit to an MND centre, and at regular intervals (4-6 months) throughout their clinical care.

In Ireland, the National MND service is a busy multidisciplinary clinic based in Beaumont Hospital. pwALS complete an ECAS with a trained neuropsychologist or research assistant at their first visit to the clinic. In this clinic, ECAS classification is based on locally validated norms which account for patient age ($\leq$ 65 years old) and level of education ($\leq$ 12 years) for all versions of the serial ECAS forms A, B and C.^{162,163} This improves the sensitivity and specificity. If patients score below a cut-off, it should trigger a referral for further in-depth neuropsychological assessment to verify cognitive status, considering premorbid IQ, age and education together.

An audit of the National MND Service between December 2012 and January 2023 examined 1,206 ECAS screening assessments. n=968 pwALS confirmed by EEC completed the ECAS at the time of the audit. Of these, n=396 pwALS completed the ECAS at T2, with n=261 of these completing the validated ECAS B form in serial order (up to January 2015; n=133 completed A-A; n=2 completed ECAS A-C). ECAS A and B serial forms have been shown to be statistically equivalent and comparable¹⁶², and cognition is not thought to fluctuate or improve over time in pwALS. Despite this, a large proportion of ECAS score classifications (Normal / Abnormal) showed a change from the Abnormal range at a patient's first visit, to the Normal range at their second visit, especially in Verbal Fluency (43.4%) and ALS-non-specific (59.2%) domains (**Table 9**). Conversely, 14.7% of pwALS who had a Normal score on the ECAS at T1, declined to Abnormal at T2, with 72%

of these (10.7% overall) displaying a clinically significant decline by the RCI. This is in contrast to Bersano et al. (2020)¹⁶⁷ who found 24% of cognitively normal patients at baseline declined on cognitive tests at follow up, with none improving.¹⁶⁷ Although some have reported that cognition declines with progressive disease stage^{164,172}, or does not improve as controls do on serial assessment⁶³⁵, many previous studies have shown few, if any, pwALS decline over time.^{169,170,636} Cognition in ALS therefore appears stable after initial assessment.

						T1-T2 Reliable Change Index (A-B)	
		Abnormal T1	Abnormal T2	Abnormal T1 & T2	Abnormal T1, Normal T2	Decline	Improve
ECAS Total	n	250/826	95/352	47/61	14/61	25/230	8/230
	%	27.39	26.42	77.05	22.95	10.9	3.48
ALS-Specific	n	215/831	93/358	46/65	19/65	25/232	4/232
	%	25.87	25.98	70.77	29.23	10.78	1.72
Verbal Fluency	n	287/952	90/387	49/87	38/87	*	*
	%	30.15	23.26	56.32	43.68		
ALS-Non-Specific	n	256/962	68/385	31/76	45/76	20/245	23/245
	%	26.61	17.66	40.79	59.21	8.16	9.39

Table 9: Proportion of ECAS Scores falling in the abnormal range, 2012-2023.

NOTE: Abnormality designated according to age and education cut-offs.¹⁶³ Clinically significant decline/improvement determined by locally validated Reliable Change Indices.¹⁶² ECAS A was administered to patients at T2 (n=133) before the validation of ECAS B, which began to be used routinely in Jan 2015. These cases were not included in the analysis. * RCI not available for individual subtests.

5.8.1 Psychometric factors

Serial administration of the same version of the ECAS (version A) introduces error by way of practice effects (PEs).^{160,161,171,635} Some find no significant practice effects on serial administration of ECAS forms (A-B-C) in healthy controls^{161,637}, but small effects have been demonstrated in other samples.¹⁶² Others have shown that healthy control performance increases from form A to B, but not from B to C when administered serially.¹⁶²

Reliable change indices (RCI) determine clinically significant declines in performance whilst also accounting for practice effects, and have been published for clinical use.^{161,162} Practice effects may account for recategorization from Abnormal to Normal at follow up. Whilst the RCI controls for these psychometric factors, environmental influences may also decrease performance at baseline (ECAS A), leading to improved performance when those factors are removed or reduced at follow-up (ECAS B).

5.8.2 Environmental factors

5.8.2.1 Distress

Given the devastating nature of an ALS diagnosis and the proximity of this diagnosis to a patient being asked to complete the ECAS at their first visit, cognitive screening at first appointment may be affected by distress. Distress is defined as internal “strain” provoked by an external “stressor” (p.219).⁶³⁸ It is a “multifactorial unpleasant experience of a psychological (i.e., cognitive, behavioural, emotional), social, spiritual, and/or physical nature” (Ownby, 2019, p.175).⁶³⁹ It is a broad label given to presenting symptoms of depression and anxiety, exists on a continuum of severity, which can differentially impact health and wellbeing, as well as cognitive testing performance.⁶³⁹ It is often measured using self-report screening tools, such as the HADS.^{580,640}

5.8.2.1.1 Causes of distress

Distress can have multiple causes in the context of the diagnosis of an incurable, life-limiting and neurodegenerative condition such as ALS. pwALS are at increased risk of mental health disorders in the periods that immediately precede and follow diagnosis.^{192,641-643} This increase in psychiatric symptoms could be part of the disease phenotype^{99,643} or could indicate emotional reactivity to symptoms and diagnosis. Rates of anxiety and depression in patients show large variability between studies, although symptoms most often appear to be mild.⁶⁴⁴⁻⁶⁴⁸

5.8.2.1.2 The cognitive impact of distress

“Anxiety can be distracting, disruptive, and incapacitating” (p. 1).⁶⁴⁹ Affective state can influence cognitive performance. Performance can decline with resource-competition between the cognitive task and distracted or anxious thoughts, although the strength of these effects can vary.⁶⁵⁰ Neuropsychological assessment should account for the effects of psychiatric and psychological symptoms due to the impact they can have on cognitive functioning and test scores.⁶⁵¹ This is not necessarily standard when administering ECAS and other screening assessments. Depressive symptoms have been shown to negatively correlate with ECAS scores, especially for executive tasks.⁶⁴⁶ The authors found no association between depressive symptoms and other cognitive measures (FAB and MoCA) nor between trait- or state-anxiety measures and ECAS scores.⁶⁴⁶ State anxiety does not correlate with individual fluctuations in cognitive performance. Instead, chronic anxiety is a better determinant and is also predictive of variable state anxiety—“trait trumps state” (Meissel and Salthouse; p.9).⁶⁵⁰

5.8.2.2 Tiredness, travel & wait times

Specialist MND centres can be regional and sometimes national—as is the case in Ireland—requiring many pwALS to travel long distances to the centre. After arrival, they can be subject to long wait times whilst waiting to see the multi-disciplinary team. Cognitive fatigue, physical tiredness and stress associated with travel and waiting in a busy out-patient clinic (e.g.: seeing pwALS further along the clinical trajectory) may also affect cognitive performance. Travelling and waiting for treatment can be a difficult experience for those with serious health conditions. In a qualitative study of patients travelling to undergo cancer treatment in Canada, themes arising from interviews included, “waiting was the most difficult part”, “travelling for treatment was distressing” and “...tiring and posed difficulties for patients”.⁶⁵²

5.8.3 The Distress Thermometer

The Distress Thermometer (DT) is a quick screening tool for subjective generalised distress. It is routinely used and well validated in clinical oncology.^{639,653} It has demonstrated utility in these settings for allocating resources and services to those most in need. The DT consists of an 11-point Likert scale, which looks like a thermometer, ranging from 0 (no distress) to 10 (extreme distress; **Figure 17**). Cut-off scores vary, however a score of above 4/10 is generally accepted as indicative of clinically significant distress, or ≥ 6 in services with more limited resources.⁶⁵⁴

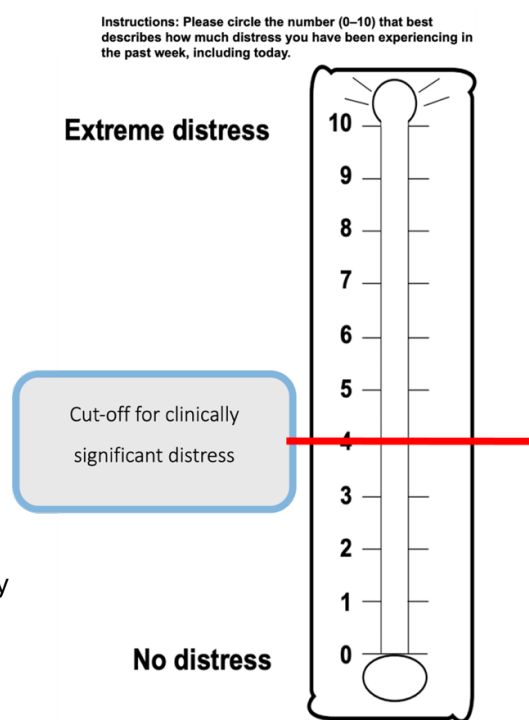


Figure 17: Distress thermometer from Ownby et al. (2019).⁶³⁹

Cut-off score of 4 based on needs within clinical oncology settings.⁶⁵⁴

5.8.4 *Study aims*

This chapter addresses **Aim 2** and **Hypotheses 2.1 to 2.4**.

The primary aim was to determine the impact of distress, behaviour, proximity to diagnosis, tiredness and distance travelled on clinic-based ECAS performance, which was hypothesised to have a negative effect (**Hypothesis 2.4**). This may warrant a change in service provision. Secondary aims were to measure the levels of distress experienced by patients in the ALS clinic and determine the factors related to distress, including behavioural change. The hypothesised effect of these variables are likely multifactorial in their impact on distress, with further distances travelled, longer wait times, closer proximity to diagnosis and higher tiredness ratings compounding, producing higher levels of self-reported distress on the DT.

5.9 Methods 2

5.9.1 *Participants*

All patients attending the National MND Clinic in Beaumont Hospital in Dublin were invited to complete an ECAS as part of their clinical care on, or as close to their first visit as possible. 72 pwALS meeting EEC for at least probable ALS completed a baseline ECAS screening assessment during the study period, 16/02/2023 to 26/10/2023. Cases excluded from this analysis included FTD diagnoses (n=1), other non-ALS MND diagnoses (PLS=1), comorbidity of other neurological conditions (CVA=1). A further 9 cases were excluded due to either incomplete ECAS (n=4) or DT (n=5) data. 60 pwALS fully completed the ECAS and DT. A subgroup of informants, who were mostly spouses/caregivers also completed the Beaumont Behavioural Inventory (BBI) and the Dimensional Apathy Scale (DAS; n=44; n=8 BBI only). All spoke English as their first language. Demographic data are shown in **Table 10**.

5.9.1.1 *Clinical categorisation*

Although a full neuropsychological assessment is needed to properly determine a patient's cognitive/behavioural status, some studies use only ECAS for its domain-based design according to the Strong Criteria (**Appendix 11**).⁵² pwALS were categorised as ALS_{ci} if they had an Abnormal ECAS Total or ECAS ALS-Specific domain scores, ALS_{bi} if they scored ≥ 7 on the BBI 'post' score or DAS total score ≥ 40 , and ALS_{cbi} if they met both criteria (see **Table 10**).¹⁶

5.9.2 *Procedure*

pwALS completed the assessment on, or close to, their first appointment as part of routine clinical care in the MND Clinic in Beaumont Hospital. All patients were administered the ECAS in-person by author (CP) and other research assistants/assistant psychologists on the clinical team. All received training on the ECAS. Companion questionnaires were completed while the patient completed the ECAS. Scores from the DT and tiredness thermometer and other study information were collected following the ECAS. Prior written informed consent was obtained from each patient as per ethics protocols, detailed in the methods section.

Table 10: Demographic variables for the pwALS at baseline cohort.

n		60
Age		63.52 (10.45)
Sex (% Female)		38.3%
Years Education		14.1 (3.4)
C9orf72 Positive (%)		12%
Cog/Beh Impairment:	ALSci	11.6%
	ALSbi*	28.3%
	ALScbi	16.67%
	ALSn	43%
Onset Site (%)	Spinal	61.7%
	Bulbar	20.0%
	Thoracic / Respiratory	3.3%
	Cognitive / Behavioural	1.7%
	Unknown	13.3%
ECAS mode of admin.	% verbal	92%
		55/60
Relation of Companion (BBI / DAS rater)	Spouse	70%
	Sibling	5%
	Parent	2.5%
	Child	22.5%

Categorisations were made based on the ECAS, BBI and DAS, they do not reflect full categorisation according to Strong Criteria (2017). ALSn=ALS without cognitive or behavioural impairment. Values presented as Mean(SD) and percentage of total (%).

* 2 cases were categorised as ALSn (based on ECAS, BBI and carer-rated DAS Total Apathy) but had abnormal carer-rated DAS Initiation Apathy scores.

5.9.3 Measures

5.9.3.1 ECAS

Participants were administered ECAS A as a baseline screening of cognitive function. Domain abnormality classifications were made based on local age- and education-adjusted normative data. 55 participants in the study completed the ECAS verbally, the remaining 5 did the written version due to bulbar dysfunction. The ECAS controls for motor dysfunction by computing a VFI which is used to score the fluency subtest based on published normative data.¹⁶³

5.9.3.2 Distress Thermometer

In the current study, pwALS were asked to rate their subjective level of distress experienced over the last week on the DT.^{639,653} High and low distress groups were created based on a cut-off score of four.^{639,653,654}

5.9.3.3 Behavioural measures

Whilst pwALS were undergoing the ECAS, companions/caregivers were asked to complete the BBI and DAS on Qualtrics using a tablet in clinic. This was most often completed by the pwALS's spouse (70%; see **Table 10**). Abnormality thresholds were defined as 2SD above the control mean, published in Radakovic et al.¹⁵⁴ (**Appendix 5**).

5.9.3.4 Other measures

A thermometer scale similar to the DT was used to quantify patients' level of tiredness at the moment of assessment (**Appendix 12**) and was administered after the ECAS and DT. Patients were also asked to report on their distance travelled to the clinic that day (km) and time of arrival at the clinic (to estimate wait times; mins). Time since MND diagnosis and other clinical information was obtained through the Irish MND Register.⁴⁶ Proximity to diagnosis was defined as follows: Recent ≤ 7 days, Mid = 7-30 days, Far ≥ 30 days.

5.9.4 Statistical analysis

To detect a small effect size ($d=0.2$) and achieve a power of 0.8 ($\beta=0.2$) with 5 predictors, a minimum sample size of 65 was required. Spearman's rank correlations and multiple linear regression were carried out to investigate the relationship between variables. As raw ECAS scores (total out of 136) were used as the dependent variable, age and education were included as covariates in all models to control for their effects. t-tests, ANOVA and Kruskal-Wallis Tests were employed to examine group differences, as appropriate (e.g.: high vs low distress, behavioural status, time since diagnosis) on ECAS domain scores. Binary logistic regression assessed the probability of membership to high/low distress groups. All test assumptions were checked, as per the methods section. Benjamini-Hochberg correction for False Discovery Rate (FDR) controlled for multiple comparisons. FDR was set as 5% for each of the models DVs (distress scores and ECAS scores). Sex can be a source of variation in rates and disclosure of mental health symptoms⁶⁵⁵, and so is included in the distress model. The multiple linear model for evaluating contributors to distress was constructed as follows:

Distress model 1: 'DT score' $\sim$ age + sex + 'recency of diagnosis' + tiredness + 'travel distance' + 'wait time'

And adding in the variable for behavioural impairment:

Distress model 2: 'DT score' ~ age + sex + 'recency of diagnosis' + tiredness + 'travel distance' + 'wait time' + 'BBI post score'

The multiple linear model for evaluating contributors to ECAS test performance was constructed as follows. Sex has been shown to have minimal effects on ECAS scores¹⁶³, and so was not included in the model for cognition:

ECAS model 1: 'ECAS Domain Score' ~ age + education + 'DT Score'

ECAS model 2: 'ECAS Domain Score' ~ age + education + 'DT Score' + 'recency of diagnosis' + tiredness + 'travel distance' + 'wait time'

5.10 Results 2:

5.10.1 Levels of distress

55% of pwALS rated their level of distress above the cut-off of 4 (Md=5) on the Distress Thermometer (DT; **Figure 18**). High distress (>4) and low distress (≤ 4) groups did not differ on any demographic variable (i.e.: sex, age, education; all $p > 0.1$).

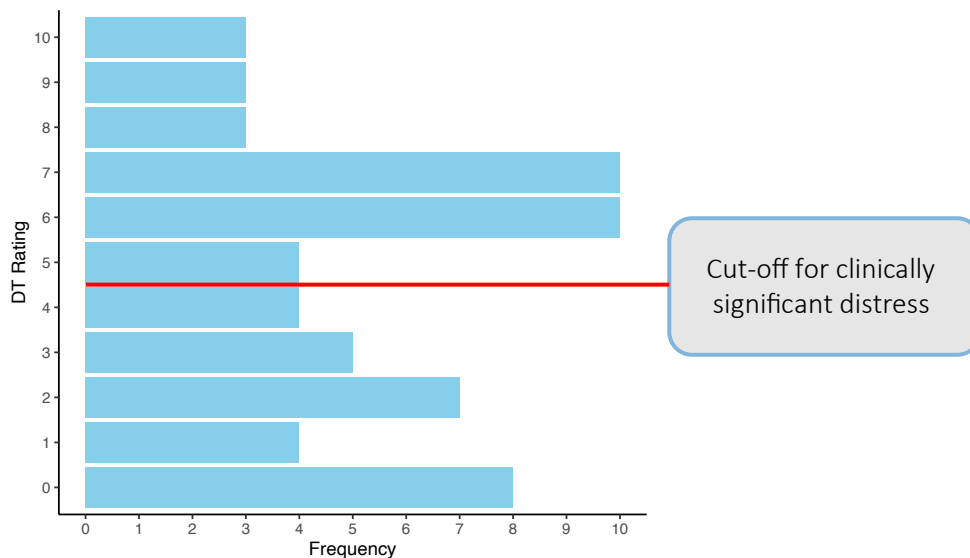


Figure 18: Frequency distribution of patient distress scores on the Distress Thermometer (DT).

The majority of cases had their first ECAS more than 30 days from ALS diagnosis date. Neither was there an association between proximity to diagnosis and endorsed distress, nor were the most recently diagnosed patients more distressed than those who'd been diagnosed longer ago.

Table 11: Distress thermometer scores by proximity to diagnosis.

Proximity to Dx	n	DT Rating
Far (>30 days)	40	5.5 (5)
Mid (7-30 days)	7	6 (2)
Recent (0-7 days)	9	5 (4)
NA ^a	5	2 (3)

^aDiagnosis date not available on MND Register.
Data presented as Median (IQR).

5.10.2 Contributors to distress

All multiple linear regression assumptions were met, however, DT residuals were not normally distributed. None of the independent level contributors could significantly predict levels of subjective distress, nor was the model as a whole significant ($p_{\text{adj}}=.3$; $\text{adj.}R^2=0.26$; **Appendix 13** shows model output and descriptive statistics). A logistic regression with distress category membership as the dependent variable was subsequently performed. No variable predicted group membership in the logistic regression, including sex (all $p>.05$).

There was no relationship between distress scores and age, sex, proximity to diagnosis or wait time (all $p>0.1$). Higher levels of tiredness correlated weakly with pwALS levels of subjective distress, but this was not a significant predictor in the regression models above ($p=.083$).

5.10.2.1 Site of onset

Bulbar onset pwALS were more likely to experience high levels of distress and to be categorised in the high distress group, although the effect was not significant (Fischer's exact tests OR: 3.1; 95%CI:[0.64,20.62]; $p_{\text{adj}}=.36$; **Table 12**).

Table 12: Rates of distress in Bulbar vs Spinal pwALS onset sites (n).

Onset Site	Low Distress	High Distress
Bulbar	3	9
Spinal	19	18

n=5 clinical onset-site data not available.

5.10.3 Distress & behaviour

To address the hypothesis that patients with behavioural impairment reported lower DT ratings caused by reduced insight, BBI (post-onset) scores were included in the logistic regression model ($n=44$). Including BBI scores improves the model ($\text{AIC}_1=75.55$, $\text{AIC}_2=69.1$), but did not predict DT group categorisation, showing a very small effect ($\beta(\text{SE})=0.016(0.02)$, $p_{\text{adj}}=.49$). Although there appeared to be more cases with mild behavioural impairment in the high- vs low-distress group, this difference was not statistically significant on a Fisher's exact test, $p=.616$ (**Table 15**).

Table 13: Distress and ECAS scores for each of the behavioural status categories on the BBI.

BBI Status	n	DT Rating	ECAS Total
Normal	26	4 (5.5)	107 (13.9)
Mild	17	6 (4)	97.8 (18.4)
Severe	9	5 (4)	93.1 (18)
NA	8	3.5 (6)	99.9 (16.2)

BBI = Beaumont Behavioural Inventory; DT = Distress Thermometer

5.10.3.1 Apathy & distress

A subset of pwALS's had carer-rated DAS data (**Table 15**). To preserve power initially, DAS apathy scores are added to a separate logistic regression to assess their relationship to distress. 22.7% of pwALS in this sample were rated as having clinically significant Total apathy (**Table 14**). Although continuous distress and apathy raw scores were not associated (all $p > .05$), those reporting high distress on the DT were 12 times more likely to be rated as having clinically significant apathy by their caregivers (**Figure 19**; $OR = 12.2$, $p_{adj} = .041$). The confidence interval is very large however (95%CI:1.42–589.04).

Table 14: pwALS grouped according to Distress and DAS Total Apathy categorisations.

	DAS Total Apathy	
	Abnormal	Normal
Low distress	1	20
High distress	9	14

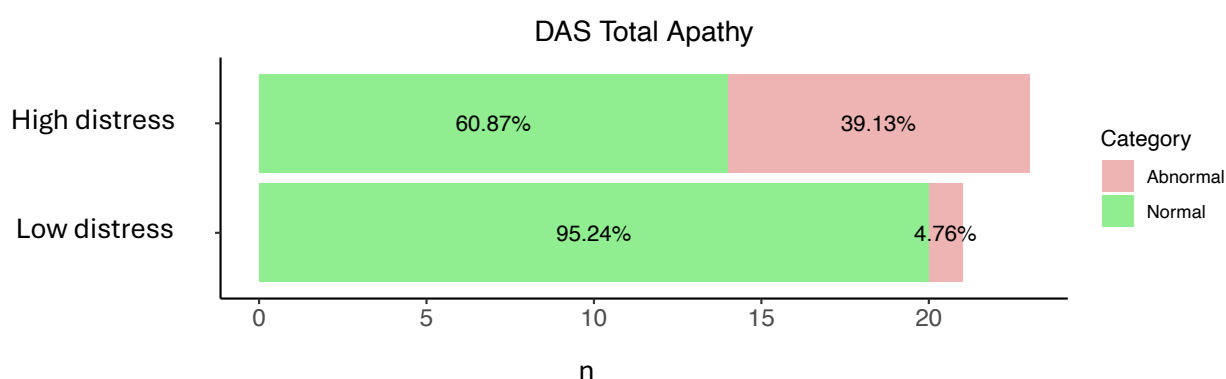


Figure 19: pwALS rated as having abnormal DAS Total Apathy were more likely to rate themselves as experiencing 'high' distress.

Table 15: ECAS, BBI & DAS mean scores & rates of abnormality.

	Whole group		High Distress		Low Distress	
	M (SD)	% Abnormal	M (SD)	% Abnormal	M (SD)	% Abnormal
ECAS (n)	60	-	33	-	27	-
ECAS Total	101.37 (16.8)	25.9%	101.97 (18.5)	25.0%	100.67 (14.8)	26.9%
ECAS ALS-Specific	75.6 (13.1)	25.9%	75.84 (14.4)	31.2%	75.22 (11.6)	19.2%
ECAS Fluency	16.45 (4.0)	21.7%	16.94 (3.7)	15.2%	15.85 (4.3)	29.6%
ALS-Non-Specific	25.82 (5.5)	20.3%	26.12 (5.6)	21.2%	25.44 (5.41)	19.2%
DAS (n)	44	-	23	-	21	-
DAS Total	29.39 (11.4)	22.7%	30.96 (14.0)	39.1%	27.67 (7.5)	4.8%
DAS Initiation	13.64 (5.0)	29.5%	13.96 (5.5)	34.8%	13.29 (4.4)	23.8%
DAS Executive	6.25 (4.7)	9.1%	7.22 (5.6)	17.4%	5.19 (3.2)	0 %
DAS Emotional	9.98 (4.2)	6.8%	10.17 (4.9)	13.0%	9.76 (3.5)	0 %
BBI (n)	52	-	29	-	23	-
BBI Post score	13.154 (16.18)	-	15.31 (19.2)	-	10.44 (11.0)	-
BBI Status:						
% Normal	-	50.0%	-	44.8 %	-	56.5 %
% Mild	-	32.7%	-	37.9 %	-	26.1 %
% Severe	-	17.3%	-	17.2 %	-	17.4 %
Tiredness ratings	3.82 (3.03)	-	4.64 (3.06)	-	2.85 (2.73)	-
Wait times	170.5 (73.24)	-	182.16 (78.56)	-	156.04 (64.68)	-
Distance travelled	111.37 (84.72)	-	112.0 (86.16)	-	110.59 (84.56)	-
Diagnostic latency (months)	13.54 (10.49)	-	15.0 (12.57)	-	11.58 (6.57)	-

5.11 Distress & cognition

Hierarchical multiple linear regression was performed to see if any predictors could account for ECAS scores above distress scores alone (**Hypothesis 2.4**). Step 1 included distress as the predictor with age and education as covariates. Patient distress was entered into the model at Step 1. Step 2 added the remaining variables of interest (i.e: duration since diagnosis, wait time, distance travelled, tiredness; hierarchical regression summary in **Table 16**). The baseline model was significant, as age and education accounted for 42% of the variance in ECAS total scores. Step 1 was not significant however, as distress ratings did not add significantly to the variance explained by the model ($\Delta R^2=0.0006$; $p>.05$). Holding age and education constant, for every unit increase in distress score, ECAS scores fell by a negligible 0.147 points. Neither was step 2 of the model significant, as adding the external factors explained no more of the variance in ECAS scores ($\Delta R^2= -0.12$; $p>.05$). **Figure 20** is a visualisation of the final model, showing

only age and education are significantly associated with ECAS Total score, holding all other predictors constant.

Each ECAS domain (ALS-Specific, Non-specific and Fluency) showed the same pattern of results. This indicates, neither distress scores, nor any other variable measured at a pwALSs' baseline visit, unduly affected ECAS scores.

Table 16: Hierarchical linear regression for ECAS Total scores and distress.

	ΔR^2	b (SE)	t	p	
Step 1:	0.0006			> .05	
Constant		110.116 (17.389)	6.333	< .001	***
Age		-0.588 (0.187)	-3.148	.003	**
Education (years)		2.094 (0.577)	3.627	.001	***
Distress Rating		-0.147 (0.621)	-0.236	.814	
	ΔR^2	b (SE)	t	p	
Step 2:	-0.012			>.05	
Constant		112.87 (21.96)	5.141	<.001	***
Age		-0.626 (0.23)	-2.748	.009	**
Education (years)		2.018 (0.71)	2.83	.007	***
Distress Rating		-0.085 (0.8)	-0.107	.916	
Duration since dx		0.014 (0.05)	0.295	.77	
Wait time		-0.002 (0.03)	-0.065	.949	
Distance travelled		0 (0.03)	0.013	.99	
Tiredness		-0.248 (0.81)	-0.305	.762	

5.11.1 *Univariate associations with cognition*

Spearman correlations found no association between Distress Thermometer (DT) scores and ECAS domain scores (all $p < 0.15$, $p > .05$). Neither did the high vs low distress groups differ from each other on ECAS domains on Wilcoxon rank sum tests with continuity correction (all $W > 400$, $p > 0.5$). Patients diagnosed within a week of their first ECAS did not differ on any ECAS domain score ($p > 0.6$) or distress scores ($p > 0.7$) from those who had been diagnosed within a month, or further away. Neither were there any associations between tiredness, distance travelled or wait times and ECAS domain score (all $p > 0.1$).

Association Between ECAS Total Score and Predictors

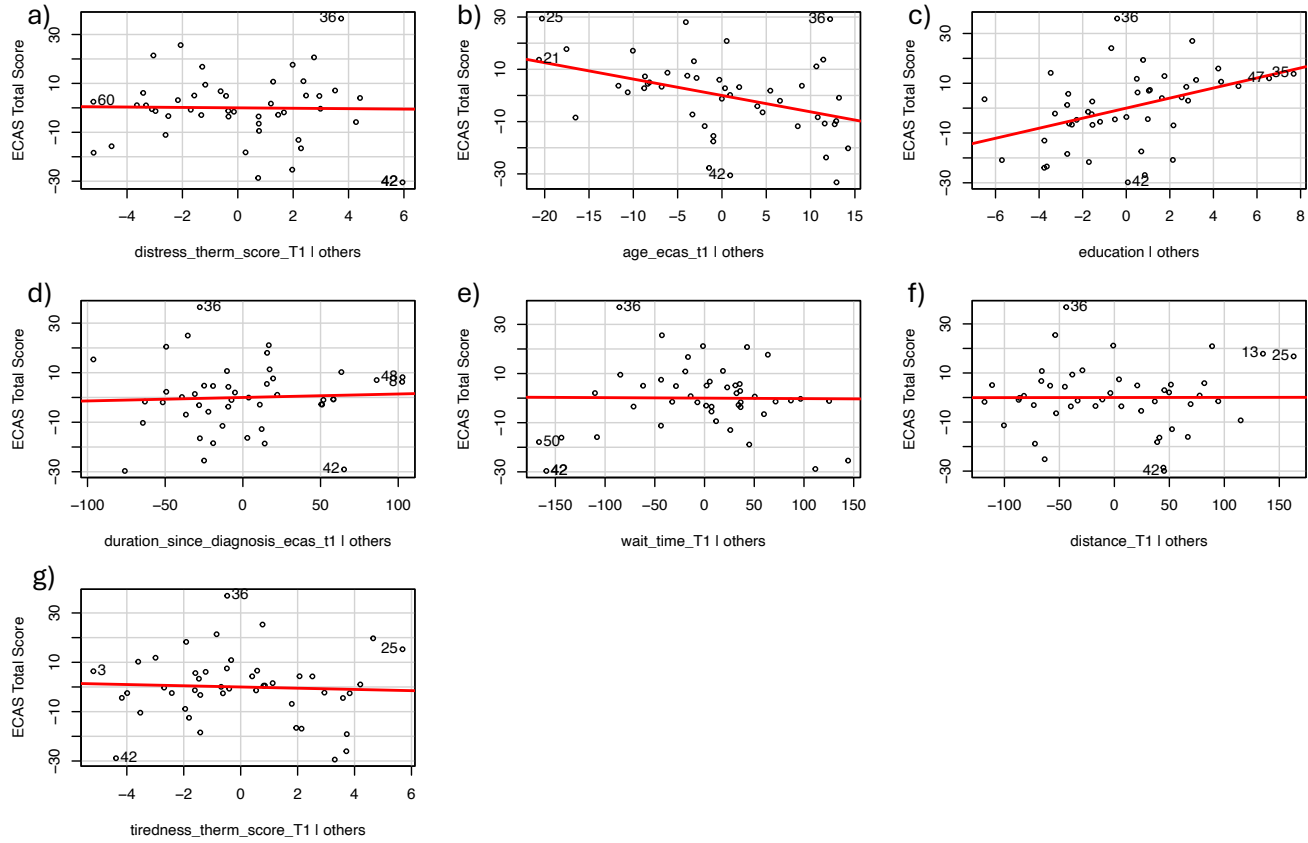


Figure 20: Added variable residual plots for predictors in the ECAS model. Only b) age and c) education are significant, holding all other variables constant.

5.12 Discussion 2

This study sought to quantify rates of distress using the DT, and evaluate if distress and other variables associated with clinic visits are impacting cognitive test scores at baseline. Over half of the pwALS in the study period were categorised as experiencing high distress (55%). Of the factors examined, including, distance travelled, tiredness, clinic wait time, proximity to diagnosis, age, and BBI scores, none were significantly related to distress, with the exception of carer-rated DAS Total Apathy scores. This agrees with previous findings on the association between physical impairment, site of onset, symptom progression rates and cognitive function.^{648,656} Site of onset may be related to increased rates of distress, however a higher-powered study will need to investigate the non-significant trend reported here. A systematic review by Fisher et al.⁶⁵⁶ found no demographic or clinical variable—including site of onset—that was predictive of distress in pwALS.

Behavioural scores and distress were not associated in this study, in contrast to work by Rabkin et al.⁶⁴⁸ who reported a strong relationship. Their study used a broader range of behavioural and cognitive measures in a large sample with a high proportion of patients exhibiting neuropsychiatric symptoms (over 68%), likely increasing sensitivity to this association.⁶⁴⁸ Consistent with this finding is that pwALS rated as displaying abnormally high apathy in the current study were twelve-times more likely (OR=12.2) to rate themselves as having high levels of distress (DT score>4). Appearing counterintuitive that overt distress is associated with affectively flat or apathetic persons, subjective awareness of an increase in apathy could explain this relationship. It suggests that apathy and distress are associated in ALS, but highlights the multitudinous factors which may contribute to endorsed distress, not fully captured here. This finding should be interpreted cautiously, as large confidence intervals reflect uncertainty, presumably caused by the small number of patients with abnormal levels of apathy endorsing low levels of distress (n=1). Together, this suggests there are individualised contributors to distress in the context of an ALS diagnosis.

5.12.1 *Practice effects on the ECAS*

27.4% of pwALS were classified as Abnormal on age- and education-matched cut-offs for ECAS Total score at baseline cognitive screening in audit presented in the introduction. This is lower but somewhat consistent with many findings reporting ALSci in approximately 35% of pwALS.^{2,16} Only 77% of cases with Abnormal ECAS Total scores at

baseline remain Abnormal at follow-up. Despite reports of no practice effects on serial administration of the ECAS (A-B)^{160,161}, and ECAS A and B being statistically equivalent¹⁶², 23% of Abnormal cases were shown here to return to the Normal range, with even higher proportions for ALS-specific (29.2%), verbal fluency (43.7%) and ALS-non-specific (59.2%) domains. This is most likely suggestive of strong practice effects, supported by the low number of cases who improved significantly by the RCI (3.48%).¹⁶² Where Costello et al.(2020)¹⁶² found this in a control population, it is demonstrated here in a prospective clinic-based population. Investigating these effects in a natural setting provides ecologically valid insights into test sensitivity and the potential for misclassification. In this clinic-based sample, just under 11% of pwALS show reliable decline from ECAS A to B, and 85.6% demonstrating stability by the RCI. These findings emphasise the importance of indices which control for practice effects for the interpretation of follow-up ECAS performance as opposed to abnormality classifications, ensuring reliability of cognitive measures over time to detect meaningful progression.

5.12.1.1 Cognitive decline in pwALS

This is one of the few studies which shows a decline in longitudinal cognitive function in ALS.^{167,171} Many others have not demonstrated decline in ECAS function over time^{166,168}, even up to 5 time points at 3-6 month intervals in the context of continued grey and white matter loss in cognitive regions of interest.¹⁶⁹ Longitudinal studies are afflicted by high attrition (~51% at 3-6 months in Tan et al.(2025)¹⁶⁹) which inhibit interpretation.^{115,172}

5.13 Study-specific limitations

The relationship between distress and tiredness may be intuitive, but could be in part due to the similar stimulus scale used to measure the two constructs. The similarity or influence of testing order on the responses in the pwALS from the DT to 'tiredness thermometer' may be the reason for this relationship. For its intended purpose, identification of people in need of support, the DT works well to identify subjective distress. But it's strength is also its limitation as the ambiguity and non-standard definition make variability a statistical challenge.

The effect of declining motor function and genetic status were not assessed here. Loss of independence, occupation and functional ability, and disclosure of a genetic and hereditary component to disease may all contribute to distress in newly diagnosed pwALS. Future studies may investigate these relationships. Whilst the aim was to test

pwALS at their first appointment in the clinic, few of the pwALS in this study completed the ECAS on the day of diagnosis. Most pwALS had their baseline screening assessment (ECAS, DAS, BBI) more than 30 days from their diagnosis date. This is likely due to two main reasons: having received a diagnosis before coming to the National Service or, being too late/tired/distressed to do the ECAS at their first visit, and so their first ECAS a follow up visit 2-6 weeks later. Given distress, or symptoms of anxiety and low mood, are generally mild around diagnosis for pwALS^{192,642,648}, often returning to baseline soon after, distress hypothesised to impact ECAS performance prior to this study may have attenuated by the time patients were tested.

5.14 Conclusion 2:

These findings suggest that external factors of attending clinic do not unduly impact ECAS scores despite high levels of subjective distress 55% in a clinic-based sample. Distress is unlikely to affect the predictive ability of the ECAS, suggesting it is a robust measure of cognitive function in the clinic. The ECAS should continue to be administered in clinic as near as possible to a patient's first visit to establish baseline cognitive function, in accordance with best practice in multidisciplinary MND care. Abnormal scores should trigger referral for extensive neuropsychological assessment.

Although distress measured by the DT is not associated with ECAS scores in this study, the frequency of elevated scores in pwALS is of concern. Distress should continue to be monitored and the DT further validated in this population so that ALS-specific cut-offs may be determined. Including the DT in routine practice, given its simplicity, would allow clinicians to quickly gauge patients' subjective well-being, ability to engage with cognitive screening and open a discussion of patient-centred needs-based clinical care. This will sign-post access to psychological, social work and other services perceived to be causing this distress for those who need it most.

5.15 Chapter conclusions

Online administration of neuropsychological assessments became commonplace, clinically, and in research studies in the wake of the Covid-19 pandemic. Part one of this chapter further supports the utility of this medium as a valid alternative to in-person assessment where needed or requested by patients and participants. The testing environment and seemingly extraneous factors around neuropsychological assessment are important considerations in the evaluation of a patient's cognitive status, as well as for carrying out research. The second study discussed in this chapter demonstrates the robustness of the ECAS as a screening tool in the face of these potential confounds. It emphasises the importance of RCIs and not abnormality criteria based on individual test administration for interpretation of serial assessment and change over time. Overall, the testing environment is important to consider in neuropsychological assessment, although the effects on test scores are likely small to negligible in the areas addressed in this chapter.

6 Chapter 6:

Results: Cognitive function in ALS kindreds – a replication, validation and longitudinal stability study

6.1 Introduction

Recently, candidate cognitive endophenotypes have been identified in unaffected relatives of pwALS, particularly in fALS kindreds.¹⁹⁴ Confrontation naming, working memory and phonemic, but not semantic, verbal fluency demonstrated the largest effect sizes above the effects of general intelligence.^{194,210,211} In his doctoral thesis, Costello²¹⁰ presents evidence that these findings meet several of Gottesman and Gould's¹⁹⁶ criteria for a putative endophenotype (p. 277; see **Table 17**). However, an important criterion for endophenotypic traits to be considered as such, is that they demonstrate temporal stability. Longitudinal assessment differentiates a stable endophenotype as a marker of genetic liability, and a biomarker of early disease/carriership.

6.1.1 *Asymptomatic C9orf72-carriers in ALS kindreds*

The effects of the identified putative cognitive endophenotypes were independent of *C9orf72*-status.¹⁹⁴ Linked to the examination of stable traits in otherwise healthy patient relatives is the progression of cognitive and behavioural change in premanifest stages of disease. Other studies of asymptomatic at-risk *C9orf72*-gene-carriers have shown mixed results on the impact of *C9orf72*. Consistent with the ALSci phenotype, decreased performance on verbal fluency tasks has been reported.^{489,508} Across studies, age appears to be a risk factor for decreased cognitive performance in *C9*-carriers compared to non-carriers.^{489,657} In FTD kindreds, *C9* carriership was associated with lifelong psychiatric/personality traits.⁹⁸ Whilst this may be evidence of early network dysfunction

in ‘presymptomatic’ individuals, it may also demonstrate stable, possibly neurodevelopmental traits associated with the expanded gene.^{81,186,467,509,517} Imaging studies show atrophy patterns longitudinally associated with *C9orf72* in pwALS and carriers who photoconvert⁸¹, but cognitive performance appears stable over a testing interval of one year.^{186,489} This interval may not be sufficient to demonstrate change in a/presymptomatic individuals.

Table 17: Endophenotype checklist according to Gottesman and Gould (2003)¹⁹⁶ criteria. From Costello (2022; p. 277, Table 10.1)²¹⁰

Endophenotype criteria	Verbal fluency	Confrontational naming	Attention to detail	Openness to experience	Conscientiousness
1) Be heritable, heritability estimate	0.52 – 0.62 (408)	0.35 (409)	0.57 ^a (410)	0.61 (406)	0.44 (406)
2) Associated with the illness	✓ (226)	✓ (411)	?	✓ (348)	?
3) Independent of clinical state	✓	✓	✓	✓	✓
4) Co-segregate within families	✓	✓	✓	✓	✓
5) Found at a higher rate in nonaffected relatives than the general population	✓	✓	✓	✓	✓

a – Heritability estimate is for the Autism Quotient total as there is no available estimate of attention to detail subscale

✓ - Evidence that this criterion is met

? – No evidence that this criterion has been met

6.1.2 Chapter aims

This chapter addresses **Aims 4** and **7**, and corresponding Hypotheses **4.1–4.4** and **7.1**. These analyses form part of the MND Family Study, which investigates cognitive endophenotypes and the effects of *C9orf72* carriership in familial ALS kindreds. Firstly, a prospective study aims to replicate the findings relating to cognitive endophenotype in fALS¹⁹⁴ in a new sample, with the same tests and procedures. Secondly, a longitudinal study aimed to, 1) assess temporal stability and state independence of candidate

cognitive endophenotypes in unaffected fALS relatives, and 2) examine the effects of *C9orf72*-carriership on cognitive performance.

A standardised difference score for verbal fluency is proposed as a more sensitive marker of the primary cognitive endophenotype, phonemic verbal fluency performance. Findings will further the understanding of endophenotypes and cognitive function in at-risk gene carriers in ALS kindreds.

6.2 Methods

6.2.1 Study Design & procedure

This chapter addresses the longitudinal and prospective arms of Phase 2 of the MND Family study, all procedures for which are outlined in the methods chapter. ECAS A was administered at baseline and B at follow-up, otherwise the battery was the same. The extended neuropsychological assessment battery is shown in **Table 6**.

6.2.2 Participants

Participants were unaffected fALS relatives and healthy controls. Recruitment flow charts are provided for Phase 1 (**Figure 21**), Phase 2 prospective replication study (**Figure 22**) and Phase 2 longitudinal study (**Figure 23**).

6.2.2.1 Attrition in the longitudinal design

55 fALS relatives did not complete full neuropsychological assessment (FB) at the time of their first ECAS (Phase 1). Nine of these completed FB in Phase 2 with ECAS T2. Participants in the final longitudinal analysis were those who completed T1 and T2. Demographic and descriptive comparisons of those who declined are evaluated.

6.2.2.2 Genetic testing

All relatives were genotyped for the *C9orf72*HRE in Phase 1. These data were not available for prospective participants recruited since 2020. Researchers were blinded to genetic status.

There were no differences between C9+ and C9- relatives on any demographic criterion (age, education, sex, premorbid IQ; all $p > 0.05$) at T1 or T2. However, many older *C9orf72*-positive cases did not opt to take part in the longitudinal study, and were more likely to decline FB in Phase 1. A number of the latter completed the FB in Phase 2, but therefore did not have longitudinal data.

6.2.3 Statistical methods

Methods to create the Relative Fluency Index are described in the methods chapter. The formula requires the correlation between phonemic and semantic fluency tests in the control sample be determined. Pearson correlation coefficients for Phase 1 and Phase 2 were $r = 0.49$ and 0.5 respectively. The pooled effect, $r_{xy} = 0.495$, is entered

into the RFI formula. Larger negative scores on the RFI indicate a larger discrepancy and worse *relative* performance on phonemic verbal fluency.

6.2.3.1 Replication: effect sizes & random-effects meta-analysis

To evaluate the consistency of group differences between Phase 1 and Phase 2 of the MND Family study, a comparison of effect sizes using random-effects meta-analysis with restricted maximum-likelihood (REML) estimation, implemented with the ‘*metafor*’ package in R (v4.8-0).^{658,659} p-values were used to assess whether the pooled effect size across samples significantly differed from zero ($\alpha = .05$). Heterogeneity was quantified using the I^2 statistic, which estimates the proportion of variance due to between-study differences rather than chance, giving a measure of the consistency. I^2 value near 0 indicates high consistency, $I^2 > 75\%$ suggests substantial inconsistency.⁶⁶⁰ While meta-analyses with only two studies are limited in precision, this approach complements p-values and aids interpretation. To directly compare effect sizes between phases, a Z-test for independent effect sizes was conducted (**Equation 11**).⁶⁵⁸ A significant z-score indicates a reliable difference in effect magnitude between phases.

Group comparisons used independent samples t-tests for normally distributed data and Wilcoxon rank-sum tests for non-parametric data. Standardised effect sizes are given as Cohen’s d , or as rank-biserial correlation coefficient (r) for non-parametric tests were calculated using pooled standard deviations. Confidence intervals were examined for overlap to evaluate consistency in replication.

Key tests of interest were those proposed as putative endophenotype from Phase 1 (FAS, DSB, BNT). Semantic fluency, RFI and WASI-II FSIQ-2 are also highlighted.

Equation 11: Formula for calculating the Z-statistic for two independent means and calculating a corresponding p-value:

$$Z = \frac{d_1 - d_2}{\sqrt{SE_1^2 + SE_2^2}}$$

Where:

- d_1, d_2 : Cohen’s d effect size from Phase 1 and Phase 2
- SE_1, SE_2 : their respective standard errors

Then, compute the p-value as:

$$p = 2 \times (1 - \Phi(|Z|))$$

6.2.3.2 *Linear mixed-effects models*

LMMs were fitted as described in the methods section. Models included random intercepts for participants, nested within families (1|family/id). This structure accounts for dependency in repeated measures and shared within-family variance, protecting against biased standard errors and inflated Type I error rates. Controls were treated as individual families. The strength of the clustering was demonstrated using ICCs, quantifying within-family similarity and the proportion of variance attributable to family-level effects. Random effects structures were tested using likelihood ratio tests.

Baseline models included fixed effects for group, time, and their interaction. Other covariates were added as appropriate, if not already accounted for by z-score standardisation. Time between assessments was evaluated case-wise for inclusion. Hierarchical addition of TOPF-FSIQ was used to examine group effects while adjusting for premorbid IQ differences. Subgroup analyses to examine effects of *C9orf72*-carriership include only fALS relatives with available genotype data. The models were structured as follows:

FB: 'Cognitive test score' ~ 'Group'*'Timepoint' + 'TOPF-FSIQ' + (1 | family/id)

FB (C9): 'Test Score' ~ 'C9 Status'*'Timepoint' + 'TOPF-FSIQ' + (1 | family/id)

Sensitivity analyses were conducted as necessary to assess the stability of model estimates. Cluster-robust variance estimation (CR2) was employed to adjust standard errors and p-values for dependency within repeated measures.^{607,608} In all models, group×time interaction was the primary parameter of interest, testing the hypothesis that temporal stability of cognitive performance is consistent across groups.

Estimated marginal means (EMMs) assessed adjusted group differences at each timepoint, accounting for covariates and random effects included in the LMM.

6.2.3.3 *Equivalency over timepoints: the TOST*

Two-one-sided t-tests (TOST) were employed to assess equivalency between baseline and follow up scores to evaluate temporal stability of the candidate cognitive endophenotypes. Rather than testing if differences between scores are statistically different from 0, as in typical hypothesis testing, the TOST assesses the probability that differences are statistically small enough to be considered meaningfully negligible.

TOST procedures included a paired t-test and two one-sided tests at pre-defined equivalence bounds. Overall equivalence is confirmed only if both upper- and lower-bounds are statistically significant. The lower-bound test is most important for the current hypothesis. Significance in this test indicates that any decline does not exceed the minimally meaningful threshold and is therefore equivalent, supporting temporal stability in fALS relatives.

Methods are based on the procedures for the ECAS validation, outlined in Costello et al. (2020)¹⁶² and use the 'TOSTER' v0.8.4 package in R.⁶⁶¹ To define a meaningful threshold, change over time (T1-T2) was calculated for controls. Equivalence bounds set a threshold of ± 0.5 standard deviations (Cohen's $d=0.5$), reflecting a small-to-moderate effect size ($\Delta M \pm 0.5(SD)$). A two-tailed alpha level of 0.05 was applied, providing power of 0.9.

Table 18a,b: Recruitment numbers and demographic characteristics of each phase of the MND Family Study.

Table 18a: Phase 1 and Phase 2 total recruitment numbers by assessment completed.

n	Phase 1 (sALS & fALS relatives)		Phase 2 (fALS relatives only)			
	ECAS	FB	T1	T2	T1	T2
			ECAS		FB	
Control	150	60	36	30	37	34
Relatives	216	149	57	52	67	42

NOTE: Final samples tested in each phase on each strand of the study. For some relatives, their T2 ECAS was a T1 FB (because they initially declined the FB in phase 1). Phase 1 shows fALS and sALS relatives combined. Phase 2 is fALS relatives only.

Table 18b: Demographic characteristics of Phase 1 and Phase 2 replication samples.

Replication Study	Phase 1			Phase 2		
	Controls	fALS Relatives	p	Controls	fALS Relatives	p
n (ECAS & FB)	60	96		37	67	-
Age	63.67 (10.2)	44.37 (17.27)	<.001	49.19 (11.59)	42.58 (13.95)	.016
Sex (% Female)	53.3%	50%	.823	56.8%	53.7%	.767
Years Education	16.42 (3.7)	16.78 (3.24)	.548	16.88 (3.33)	17.17 (3.01)	.648
C9+ gene carrier n (%)	-	16 (17%)	-	-	*	-
C9 family n (%)	-	44 (45.8%)	-	-	*	-
fALS family status ^a (%)						
Definite	-	23.5%	-	-	79%	-
Probable	-	14.8%	-	-	9%	-
Possible	-	27.5%	-	-	12%	-

NOTE: Scores are given as Mean (SD), unless otherwise stated. sALS relatives not included: n = 53, 34.2% of total relatives group.* Genotype data not available for prospective participants at the time of writing.

Figure 21: Flow-chart for Phase 1 recruitment (by Costello & Ryan^{210,211})

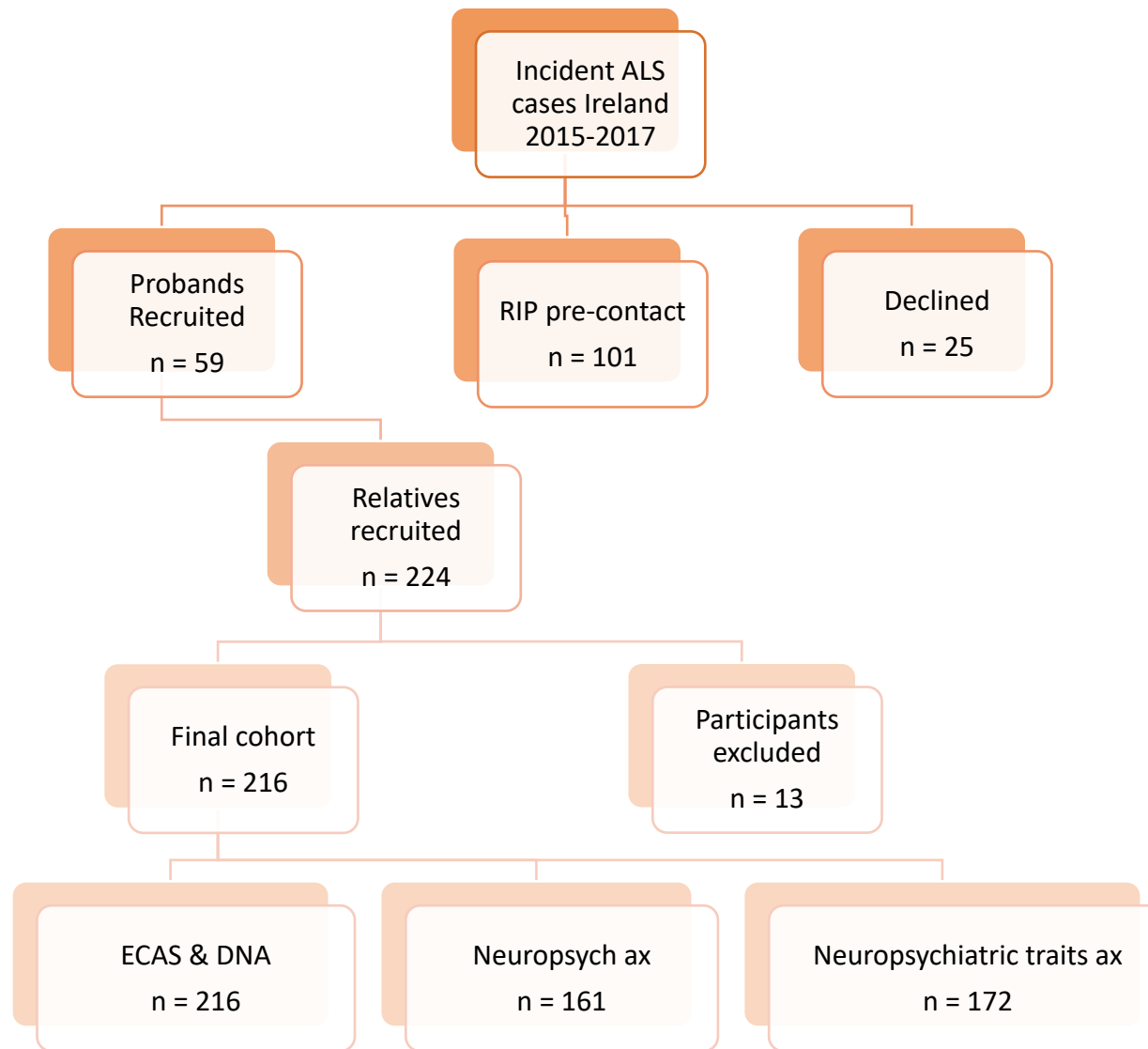


Figure 22: Flow-chart for Phase 2 recruitment of prospective replication sample.

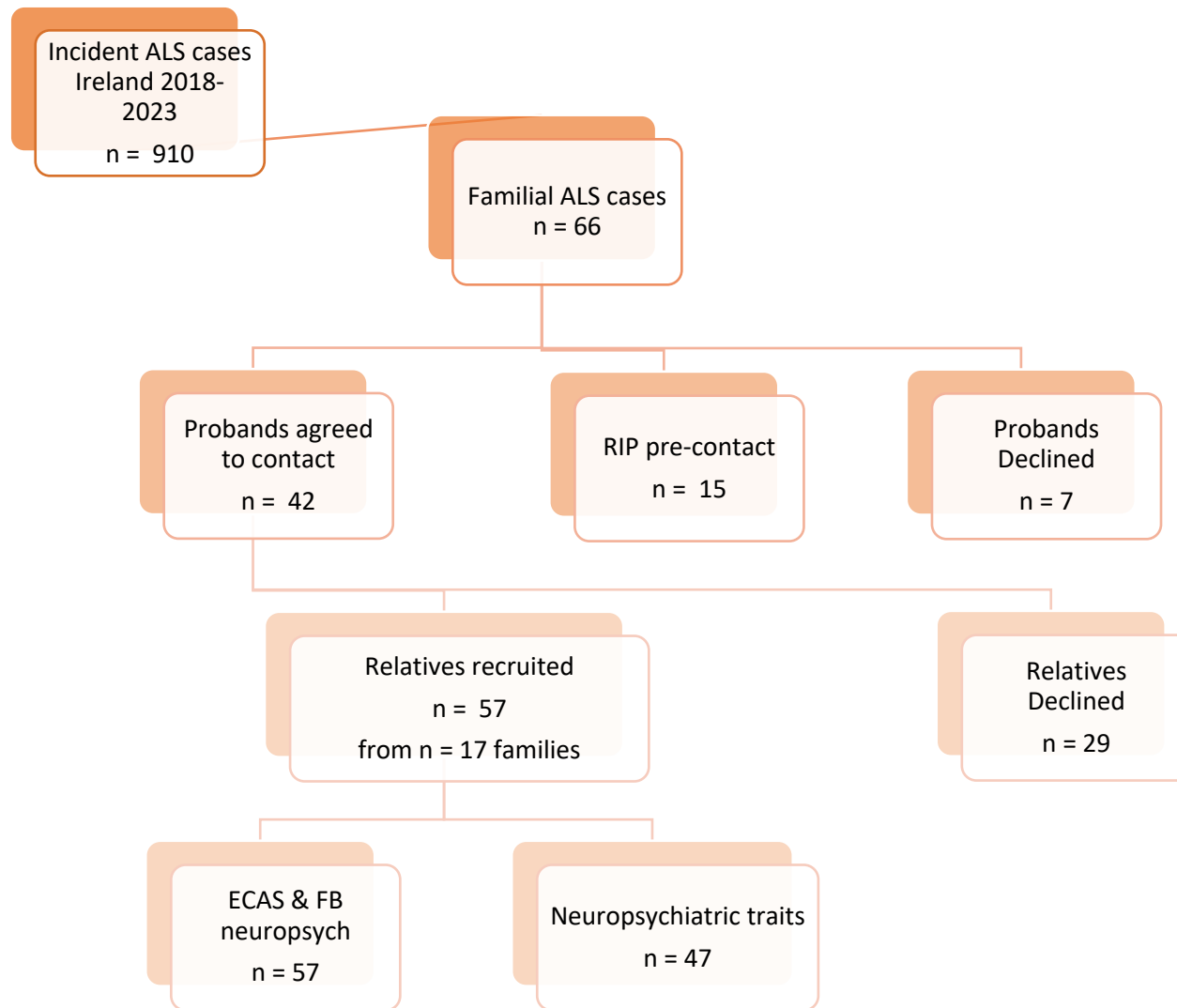
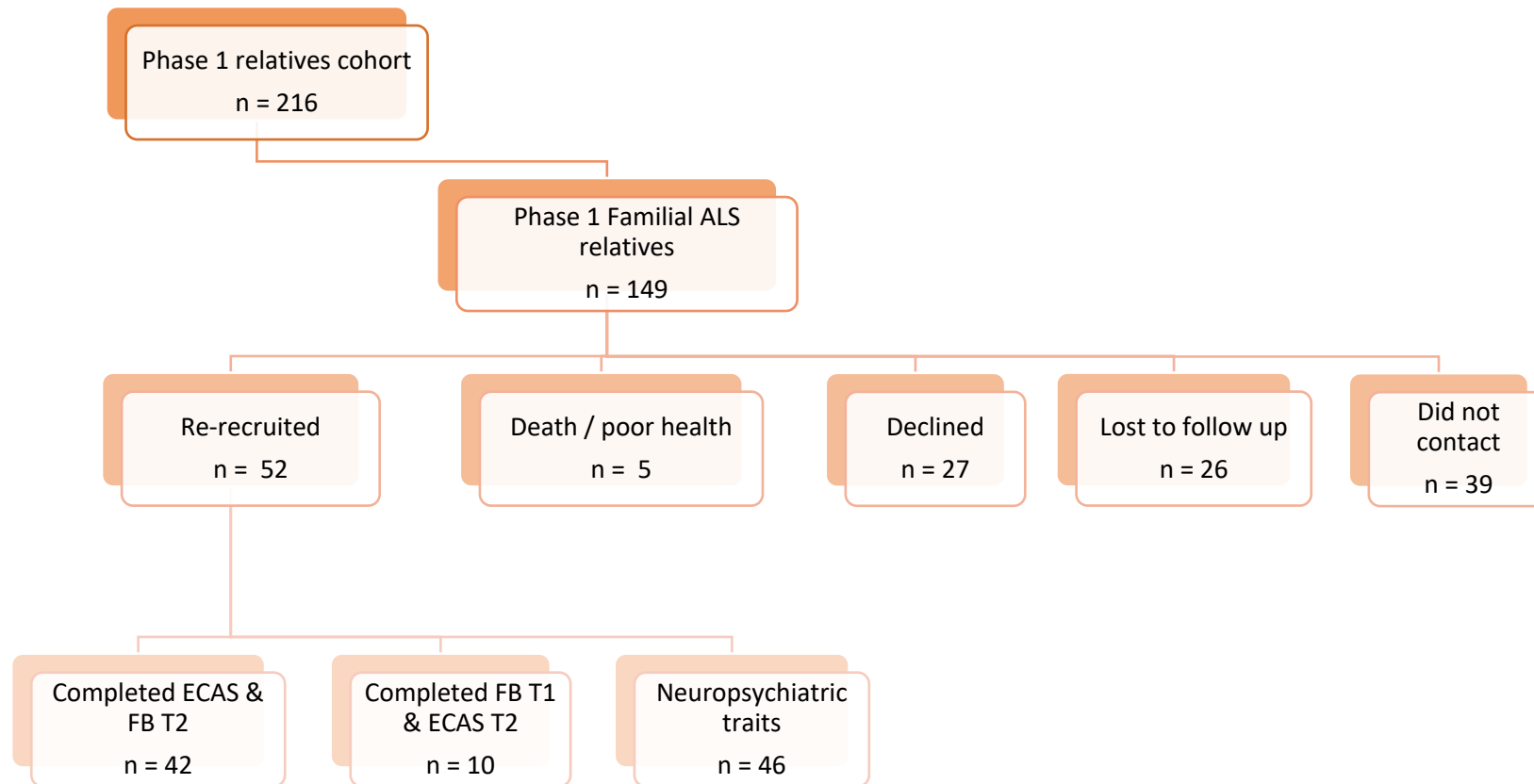


Figure 23: Flow-chart for Phase 2 recruitment of longitudinal study sample.



6.3 Results

6.3.1 Prospective replication study

Table 19 and **Table 20** detail the results of the replication study. Groups were well matched for sex and education in both phases. Phase 2 groups were better matched than Phase 1 for premorbid IQ estimates ($p=.529$) and age, but fALS relatives were still significantly younger than control participants ($p=.02$).

6.3.1.1 Replication: Random-effects meta-analysis

Effect sizes for between group differences in Phase 1 were large on key tests, as seen in **Figure 24** and **Table 19**. fALS relatives scored significantly lower than controls on several key tests. In decreasing order of effect size: phonemic verbal fluency (FAS; $d=0.86$, $p<.001$), working memory (DSB; $d=0.75$, $p<.001$), confrontation naming (BNT; $d=0.67$, $p<.001$) as well as current general IQ (WASI-II FSIQ-2; $d=0.46$, $p=.009$). Other tests showed large effects, but were not independent of general intelligence.²¹⁰

These same key variables did not show statistically significant effects in the smaller Phase 2 sample, with the exception of confrontation naming (BNT), which showed equivalent effect sizes and significant group differences ($d=0.6$, $p=.007$). Effect sizes for the working memory task and current IQ estimates were smaller but in the same direction as Phase 1 (DSB: $d=0.33$, $p=.134$; WASI-II: $d=0.28$, $p=.161$). Notably, phonemic verbal fluency did not replicate the effects shown in Phase 1. Scores were not lower than controls, as they had been in Phase 1. In Phase 2 FAS performance did not differ statistically, and effect sizes were very small, with wide confidence intervals ($d=0.1$, 95%CI:[-0.31,0.51], $p=.643$). Conversely, there were significant medium effects for higher semantic fluency ($d=-0.47$, 95%CI:[-0.88,-.06], $p=.019$) and Digit Span Forwards ($d=0.83$, $p<.001$) performance by fALS relatives. These were not evident in the Phase 1 cohort.

Despite the smaller effects shown in Phase 2, acceptable heterogeneity ($I^2<0.75$) was found for confrontation naming (BNT), working memory (DSB) and current IQ estimates (WASI-FSIQ-2), with small to medium effect sizes ($d_{\text{pooled}}>.38$). The pooled effect sizes ('Estimate') were significantly larger than 0 for each of these tests, but FAS was not significant ($d_{\text{pooled}}=0.487$, 95%CI:[-0.26,1.23], $I^2=87.1\%$, $p=.201$; **Table 19**), indicating that effect sizes were inconsistent between phases.

There were no group differences in HADS anxiety levels in either phase ($p>.05$). fALS relatives had higher levels of depressive symptoms in Phase 1, with small effect sizes

($d = -0.27$, $p = .004$) which were not present in Phase 2 ($I^2 = 77.24\%$, $p = .535$). Spearman correlations found no relationship between mood scores and cognitive variables (all $p_{\text{adj}} > .05$). GHQ scores showed the opposite pattern, but differences did not reach significance at either phase ($p > .05$).

6.3.1.2 Replication: Z-test

These findings are supported by the results of the z-test. Inconsistent effect sizes were found for the two verbal fluency tasks across phases (FAS: $z = 2.78$, $p = .005$; Semantic/Category: $z = 2.47$, $p = .014$). Significantly different effect sizes were also evident for verbal and visual memory, and digit span forwards (all $|z| > 2.4$, $p < .016$). Z-test results and change directions for inconsistent effects are shown in **Table 20**.

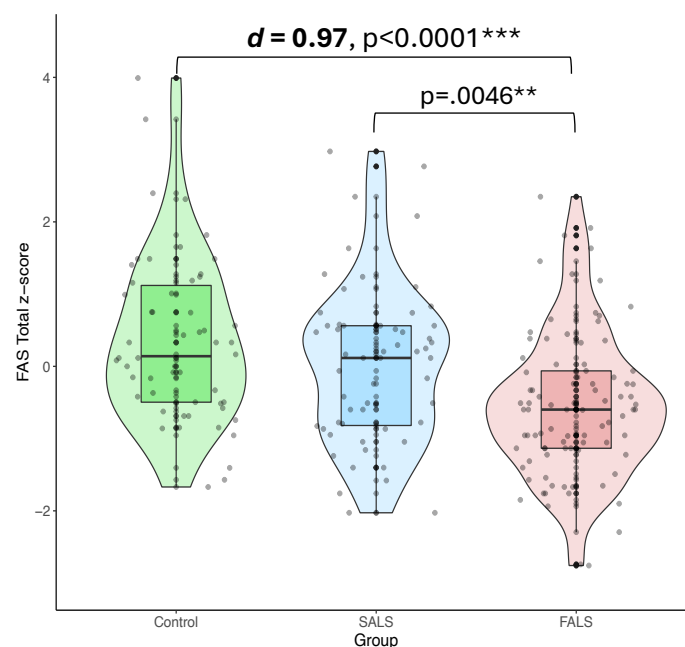


Figure 24: Comparison of controls and the two unaffected relatives groups of sporadic (sALS) and familial (fALS) pwALS in Phase 1.

NOTE: Differences between controls and fALS relatives showed a very large effect size on phonemic verbal fluency.

Table 19: Extended neuropsychological assessment results from Phase 1 and Phase 2 replication samples.

	Phase 1: T1		Phase 2: T1		Phase 1		Phase 2		Phase 1 vs 2		
	Controls	fALS ^e Relatives	Controls	fALS Relatives	d/r ^a [95%CI]	p ^a	d/r ^b [95%CI]	p ^b	Estimate [95%CI]	f ²	p
n	60	96	37	67	-	-	-	-	-	-	-
Age	63.67 (10.2)	44.37 (17.27)	49.19 (11.58)	42.58 (13.95)	1.28 [0.92,1.63]	<.001*	0.45 [0.04,0.86]	.021*	-	-	-
Sex (% Female)	53.3%	50%	56.8%	53.7%	-	-	-	-	-	-	-
Years Education	16.42 (3.7)	16.78 (3.24)	16.88 (3.33)	17.17 (3.01)	-0.1 [-0.43,0.22]	.541	-0.13 [-0.53,0.28]	.557	-	-	-
C9+ gene carrier [n (%)]	-	16 (17%)	-	- ^d	-	-	-	-	-	-	-
C9 family	-	44 (45.8%)	-	- ^d	-	-	-	-	-	-	-
TOPF-FSIQ (Std. Score)	110.22 (12.4)	104.25 (12.34)	112.19 (9.62)	110.5 (11.54)	0.48 [0.15,0.81]	.005*	0.12 [-0.28,0.53]	.529	0.321 [-0.03,0.67]	43.77	.069
Primary tests of interest											
WASI-FSIQ-2 (Std. Score)	105.26 (16.1)	98.23 (14.43)	111.25 (10.59)	107.41 (13.72)	0.46 [0.12,0.79]	.009*	0.28 [-0.14,0.69]	.161	0.385 [0.13,0.65]	0	.004*
FAS Total	0.36 (1.2)	-0.51 (1.03)	-0.12 (1.07)	-0.22 (0.97)	0.86 [0.51,1.21]	<.001*	0.1 [-0.31,0.51]	.643	0.487 [-0.26,1.23]	87.1	.201
DS-Backward span	0.72 (1)	-0.01 (1.01)	0.81 (1.12)	0.5 (0.86)	0.75 [0.4,1.08]	<.001*	0.33 [-0.07,0.74]	.134	0.555 [0.15,0.96]	56.92	.007*
BNT Spontaneous	-0.19 (0.7)	-1 (1.44)	0.21 (0.73)	-0.25 (0.76)	0.67 [0.33,1.0]	<.001*	0.6 [0.19,1.02]	.007*	0.574 [0.31,0.84]	0	<.001*
Category Fluency	-0.03 (1.1)	-0.2 (0.86)	-0.07 (0.93)	0.41 (1.1)	0.19 [-0.13,0.52]	.281	-0.47 [-0.88,-.06]	.019*	-0.126 [-0.77,0.52]	83.58	.703
RFI score	0.38 (1.19)	-0.21 (1.14)	-0.05 (1.0)	-0.63 (1.38)	0.51 [0.19,0.82]	<.001*	0.46 [0.05,0.87]	.017*	0.488 [0.24,0.74]	0	<.001*
Other cognitive tests											
RMET	-0.17 (0.9)	-0.11 (1.1)	0.36 (0.8)	0.22 (0.79)	-0.05 [-0.37,0.28]	.765	0.15 [-0.27,0.56]	.496	0.027 [-0.23,0.28]	0	.838
Immediate story memory	0.18 (0.9)	0.36 (0.92)	0.72 (0.64)	0.74 (0.76)	-0.18 [-0.52,0.16]	.291	-0.07 [-0.49,0.34]	.726	-0.138 [-0.4,0.12]	0	.303
Delayed story memory	0.73 (1.1)	0.15 (0.93)	0.72 (0.81)	0.61 (0.86)	0.6 [0.26,0.94]	.001*	0.12 [-0.29,0.54]	.551	0.376 [-0.09,0.84]	66.99	.114

	Phase 1: T1		Phase 2: T1		Phase 1		Phase 2		Phase 1 vs 2		
	Controls	fALS ^e Relatives	Controls	fALS Relatives	d/r ^a [95%CI]	p ^a	d/r ^b [95%CI]	p ^b	Estimate [95%CI]	I ²	p
RAVLT Total	1.08 (1.3)	0.36 (1.23)	0.64 (0.48)	0.61 (0.74)	0.57 [0.24,0.91]	.001*	0.17 [-0.28,0.62]	.501	0.399 [0.01,0.79]	49.64	.046*
RAVLT Delayed recall	0.65 (1.5)	0.02 (1.15)	0.39 (0.94)	0.54 (0.83)	0.49 [0.16,0.83]	.006*	-0.2 [-0.65,0.25]	.422	0.165 [-0.51,0.84]	82.83	.632
RAVLT recognition	0.56 (0.9)	0.34 (1.26)	0.64 (0.48)	0.61 (0.74)	0.10 ^c [-0.10,0.29]	.334 ^c	-0.1 ^c [-0.35,0.15]	.775 ^c	0.098 ^c [-0.18,0.37]	0	.481
RCFT immediate	0.91 (1.6)	0.02 (1.55)	0.53 (1.33)	0.71 (1.13)	0.55 [0.21,0.89]	.002*	-0.15 [-0.56,0.26]	.496	0.211 [-0.48,0.9]	84.92	.547
RCFT delayed	0.54 (1.5)	0.04 (2.82)	0.45 (1.26)	0.57 (1.28)	0.43 [0.09,0.78]	.01*	-0.09 [-0.5,0.32]	.675	0.186 [-0.33,0.7]	72.87	.475
DS-Forward span	0.35 (0.9)	0.23 (0.95)	0.94 (0.99)	0.12 (1.0)	0.14 [-0.18,0.47]	.386	0.83 [0.4,1.24]	<.001*	0.471 [-0.2,1.14]	84.28	.168
CWIT inhibition errors	0.41 (0.6)	0.14 (0.73)	0.31 (0.62)	0.33 (0.53)	0.25^c [0.07,0.42]	.008 ^c *	-0.016 ^c [-0.25,0.22]	.878 ^c	0.193 ^c [-0.25,0.64]	65.46	.396
CWIT inhibition time	0.23 (0.8)	-0.07 (1)	0.47 (0.66)	0.3 (0.77)	0.33 [-0.01,0.66]	.045*	0.2 [-0.21,0.61]	.32	0.278 [0.02,0.54]	0	.035*
CWIT switching errors	0.16 (0.7)	0.07 (0.78)	0.44 (0.41)	0.43 (0.41)	0.08 ^c [-0.11,0.27]	.366 ^c	0.001 ^c [-0.24,0.23]	.972 ^c	0.079 ^c [-0.18,0.34]	0	.548
CWIT switching time	0.24 (0.9)	-0.02 (0.95)	0.35 (0.94)	0.3 (0.76)	0.17 ^c [-0.01,0.36]	.061 ^c	0.07 [-0.34,0.47]	.764	0.209 ^c [-0.05,0.47]	0	.113
HADS anxiety (raw score)	3.82 (2.5)	4.67 (3.36)	4.5 (2.76)	4 (2.77)	-0.11 ^c [-0.31,0.09]	.261 ^c	0.10 ^c [-0.2,0.42]	.546 ^c	-0.105 ^c [-0.53,0.32]	39.9	.627
HADS depression (raw score)	0.94 (1.4)	1.98 (2.22)	2.13 (2.25)	1.75 (2.07)	-0.27^c [-0.46,-.09]	.004 ^c *	0.18 ^c [-0.2,0.44]	.569 ^c	-0.226 ^c [-0.94,0.49]	77.24	.535
GHQ (raw score)	10.04 (2.7)	10.56 (4.65)	11 (7.3)	19.31 (15.38)	0.023 ^c [-0.18,0.23]	.820 ^c	-0.28 ^c [-.59,0.04]	.091 ^c	-0.287 ^c [-0.7,0.13]	34.68	.177

^a Phase 1 between group; ^b Phase 2 between groups; ^c non-parametric data. Wilcoxon rank-sum test and rank-biserial correlation coefficient for effect size shown here. ^d Genotype data not available for prospective participants in Phase 2 at time of writing. ^e n=53 SALS relatives not included.

NOTE: Cognitive scores are z-scored and presented as 'Mean (SD)', unless otherwise stated. Effect sizes and p-values are unadjusted.

d/r = effect size: Cohen's d / rank-biserial correlation coefficient. **Estimate** = pooled effect size across phases (d/r). **I²** = percentage heterogeneity between samples. **p** = pooled effect size estimate significantly different from 0. **RFI** = Relative Fluency Index.

Table 20: Results of the z-test testing whether effect sizes in Phase 2 are significantly different from Phase 1.

	z	p	Consistent	Effect size change direction
Primary tests of interest				
WASI-FSIQ-2	0.665	.506	✓	-
FAS Total	2.784	.005	✗	Decreased
DS-Backward span	1.523	.128	✓	-
BNT Spontaneous	0.939	.348	✓	-
Semantic Fluency	2.469	.014	✗	Increased
RFI score	0.193	.847	✓	-
Other cognitive tests ^a				
RMET	-0.717	.473	✓	-
Immediate story memory	-0.399	.69	✓	-
Delayed story memory	1.745	.081	✓	-
RAVLT Total	1.41	.159	✓	-
RAVLT Delayed recall	2.416	.016	✗	Decreased
RCFT immediate	2.572	.01	✗	Decreased
RCFT delayed	1.92	.055	✓	-
DS-Forward span	-2.522	.012	✗	Increased
CWIT inhibition time	0.47	.638	✓	-

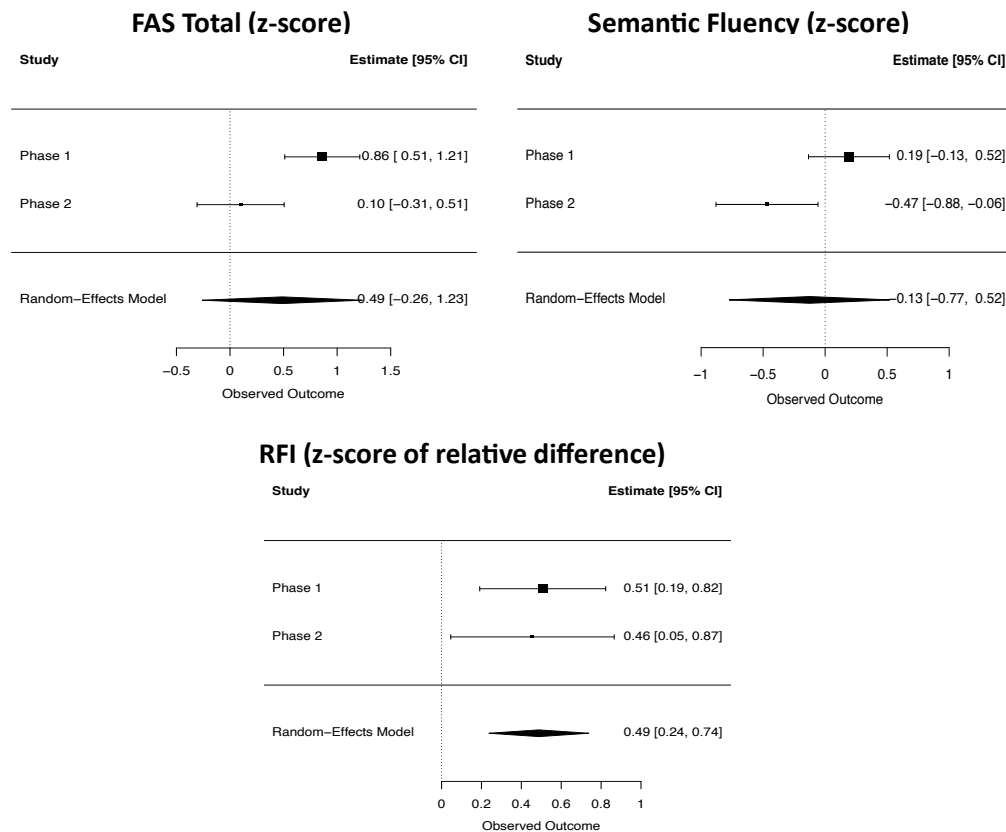
^a z-test statistic cannot be calculated for non-parametric data. These tests are omitted.

NOTE: uncorrected p-values show whether differences in effect sizes between Phase 1 and Phase 2 are statistically significant. Non-significant results indicative of no difference and interpreted as a consistent replication of the effect. Indications of consistency between phases and direction effect (*d*) changed if not consistent.

6.3.1.3 Replication: RFI score performance

Relative Fluency Index (RFI) scores are consistent in Phase 1 and 2, with medium effect sizes, highlighting consistency for discrepant performance on semantic and phonemic verbal fluency tasks for unaffected fALS relatives. The I^2 statistic in the random-effects meta-analysis (**Table 19**) indicates very low heterogeneity, with a medium and statistically significant pooled effect size ($d_{\text{pooled}}=0.562$, $p < .001$) and the z-test indicates that effects sizes in Phase 1 and 2 were not significantly different ($z=0.589$, $p=.556$; **Table 20**). In Phase 1, the RFI has a lower effect size between groups ($d=0.51$ 95%CI:[0.28,0.97]) than FAS alone however ($d=0.86$, 95%CI:[0.51,1.21]), likely as the introduction of more error due to the combination of measurements. Forest plots show the effect sizes between groups across phases (**Figure 25**). Cumulative distribution for RFI z-scores is provided in **Appendix 16**.

Figure 25: Forest plots showing Cohen's d effect sizes for Phase 1 and 2 verbal fluency outcomes.



NOTE: Depicts consistent relative effect sizes on each fluency task (phonemic [FAS] and semantic) and the RFI across phases. z-scores account for age and education.

6.3.2 Longitudinal stability study

6.3.2.1 Sample characteristics

Baseline demographics are shown in **Table 21**. Groups were matched for sex ($p=.616$), years of education ($p=.195$) and the interval between testing ($p=.109$), but controls were significantly older than the relatives ($t(71.37)=2.98$, $p=.004$) and had higher baseline TOPF-FSIQ ($t(71.8)=2.42$, $p=.018$; **Figure 26**). Mean neuropsychological test scores across timepoints, and mean change scores over time are shown in **Appendix 15**. **Figure 27** is a visual representation of these data in the key cognitive domains.

Table 21: Baseline sample characteristics for longitudinal study

Longitudinal FB sample		Controls	fALS Relatives	p
n		32	42 ^b	-
Age M (SD)		54.13 (11)	43.26 (15.5)	.004*
Sex (% Female)		56.2%	47.6%	.616
Years Education M (SD)		16.58 (2.8)	17.46 (2.9)	.200
TOPF-FSIQ T1		111.3 (9.1)	105.53 (11.4)	.018*
Interval Md (IQR)		46.5 (18.7)	45.9 (35.2)	.109
C9+ Gene carrier		-	10 (23.8%)	-
C9 family		-	23 (54.5%)	-
fALS family status ^a	Definite	-	33.3%	-
	Probable	-	16.7%	-
	Possible	-	50.0%	-
Distinct families (n)		31	14	-

^a By the Byrne Criteria. ^{108,662} ^b n=9 (n=6 C9+) fALS relatives completed ECAS T1 and T2 but only have baseline (T1) extended battery (FB) data.

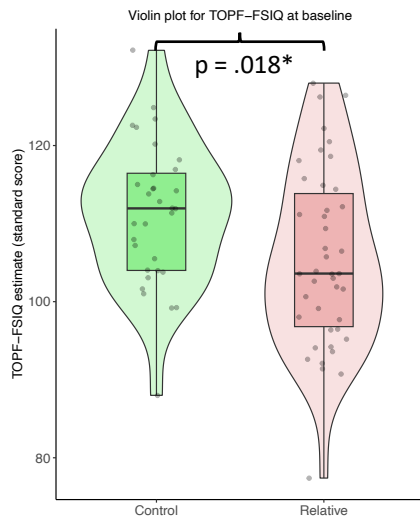


Figure 26: Violin plot showing discrepancy in TOPF-FSIQ premorbid IQ estimates at baseline in the longitudinal study sample.

6.3.2.2 Evaluating attrition bias

In order to address potential attrition bias in the longitudinal design, baseline characteristics of participants who declined repeat assessment were compared with those who completed T2. Decliners did not differ in terms of genetic status or age, but were significantly less educated ($p < .05$). They also had poorer ECAS and confrontation naming performance at baseline. Participants with abnormal ECAS Total scores were 4.8-times more likely to decline participation in Phase 2 (Fisher's exact test: $OR = 4.75$, $p = .025$). Full results are provided in **Appendix 14**.

6.3.2.3 *Linear mixed-effects models*

Hierarchical linear mixed-effects regressions were run for each primary neuropsychological variable of interest with random intercepts for participants nested within families. Model comparisons using Likelihood Ratio Tests (LRTs) showed that the inclusion of family in the random-effects structure did not significantly improve any of the models, suggesting low family-level variability after accounting for individual-level random intercepts. Nonetheless, it was important to control for the dependence of family in this design, and therefore family and individual random effects were retained for consistency. TOPF-FSIQ was added hierarchically to test whether fALS relatives scored lower than controls over time after adjusting for premorbid IQ estimates. The group×time interaction term was the primary parameter of interest.

6.3.2.3.1 *LMM: Phonemic verbal fluency (FAS)*

There was low clustering within family for phonemic verbal fluency scores (ICC=0.145). A LRT showed that including testing interval as a fixed effect did not improve fit ($p=.959$), and was not retained. Model diagnostics found two high-leverage cases (one relative and one control). Sensitivity analysis removed these cases, and re-ran the models. This negligibly changed most model parameters, although timepoint became a significant predictor, showing FAS z-scores increased significantly from T1 to T2 (CR2 robust standard errors applied: $B(SE)=0.33(0.13)$, $p_{CR2}=.019$; **Table 22**). All other model assumptions are met.

In Step 1, group is a significant predictor of age- and education-stratified FAS z-scores, controlling for random cluster effects. It is no longer significant with the addition of TOPF-FSIQ in Step 2, suggesting premorbid IQ accounted for group differences (**Table 22**).

TOPF-FSIQ was the only significant fixed effect in the final model ($\beta=0.38$, $p=.001$). The main effects of time showed a positive trend ($p=.138$), which was significant in the sensitivity analysis ($p_{CR2}=.019$). Although there was a trend for relatives to score $|z|=0.33$ lower than controls, holding TOPF-FSIQ constant, this did not reach significance ($p=.248$). Crucially, there were no significant interaction effects of group and time at Step 1 ($\beta=0.14$, $p=.47$) or Step 2 ($\beta=0.13$, $p_{CR2}=0.97$). Random intercepts indicated greater variance at the individual (SD=0.73) than the family level (SD=0.41), suggesting that most of the variability in FAS scores is attributable to differences between individuals rather than clustering within families.

Table 22: Hierarchical linear mixed-effects model table for phonemic verbal fluency (FAS Total z-score – relatives vs controls).

FAS Total (z-score)	B(SE)	df	t	β	p	
Step 1						
Fixed effects						
Constant	0.08 (0.2)	103.74	0.382	0.18	.704	
Group	-0.61 (0.26)	32.59	-2.326	-0.54	.026*	
Timepoint (T1-T2)	0.23 (0.16)	70.85	1.449	0.21	.152	
Interaction (Group $\times$ Time)	0.15 (0.21)	70.1	0.727	0.14	.470	
Step 2						Robust SE p_{CR2}^a
Fixed effects						
Constant	-4.36 (1.29)	52.85	-3.393	0.06	.001	.017*
Group	-0.33 (0.28)	41.08	-1.172	-0.29	.248	.180
Timepoint (T1-T2)	0.24 (0.16)	70.71	1.5	0.22	.138	.019*
Interaction (Group $\times$ Time)	0.14 (0.21)	69.9	0.686	0.13	.495	.969
TOPF-FSIQ	0.04 (0.01)	51.95	3.488	0.38	.001	.013*
Random effects				SD		
ID within Family				0.733		
Family				0.407		
Residual				0.621		

^a Robust p-values (p_{CR2}) calculated using cluster-robust standard errors (CR2).

NOTE: Table shows unadjusted coefficients. Age and education accounted for by z-scores.

6.3.2.3.2 LMM: Digit span backwards

There was a weak clustering within family for digit span backwards (DSB) scores (ICC=0.104). Model diagnostics confirmed all assumptions were met.

Group was significant in Step 1 ($p=.026$). The addition of TOPF-FSIQ in Step 2 accounted for the between-group variance, so that TOPF-FSIQ was the only significant fixed effect in the final model ($p<.001$; **Table 23**), including the interaction term ($\beta = -0.07$, $p=.758$). Standardised beta coefficients for group were relatively large in the final model, indicating that fALS relatives scored on average 0.35 points lower than controls on the DSB, but did not reach significance ($B(SE) = -0.35(0.22)$, $\beta = -0.34$, $p=.105$). The random effects of family reduced to 0 (returned a singular fit) in Step 2, indicating that the random effects structure was overparameterised given the observed data. This indicates that variance is negligible at the family level and is not contributing to the model above that of the random effects of individual ($SD=0.49$; **Table 23**).

Table 23: Linear mixed effects model for Digit Span Backwards (DSB) for relatives and controls over two timepoints.

Digit span backwards	B(SE)	df	t	β	p
Step 1					
Fixed effects					
Constant	0.68 (0.18)	96.95	3.795	0.25	<.001*
Group	-0.61 (0.26)	33.21	-2.332	-0.59	.026*
Timepoint (T1-T2)	0.2 (0.19)	71.55	1.058	0.19	.294
Interaction (Group × Time)	-0.08 (0.25)	71.17	-0.315	-0.08	.754
Step 2					
Fixed effects					
Constant	-4.02 (0.92)	71.97	-4.386	0.12	<.001*
Group	-0.35 (0.22)	127.85	-1.635	-0.34	.105
Timepoint (T1-T2)	0.2 (0.19)	71.79	1.052	0.19	.296
Interaction (Group × Time)	-0.08 (0.25)	71.35	-0.31	-0.07	.758
TOPF-FSIQ	0.04 (0.01)	70.21	5.218	0.44	<.001*
Random effects				SD	
ID within Family				0.490	
Family				0	
Residual				0.743	

NOTE: Table shows unadjusted coefficients. Age accounted for by z-scores. Education did not improve model above that of TOPF-FSIQ.

6.3.2.3.3 LMM: Confrontation naming

There was very weak clustering within family for the relatives on confrontation naming (BNT) scores (ICC=0.053). A LRT including testing interval as a fixed effect confirmed an improved model fit ($p=.959$), and so was retained ($p<.001$). Model diagnostics showed small evidence of heteroscedasticity (Breusch-Pagan test: $p=.025$). Cluster-robust standard errors were therefore employed to interpret the model. One case (C9+) had high leverage over the model. Removing this case in a sensitivity analyses did not change conclusions, although the interaction term became non-significant ($p=.061$).

Step 1 found interval (months) had a small but significant effect; naming scores decreased by 0.012 points for ever unit increase in interval (CR2 applied: $B(SE) = -0.009(0.003)$, $p_{CR2}=.012$). **Table 24** shows that the intercept (constant), group and the interaction term were also significant predictors of confrontation naming scores, but timepoint was not ($p=0.155$). Outcomes remained the same in Step 2. TOPF-FSIQ also demonstrated a significant fixed effect on BNT scores, which increased 0.0174 points for

every unit increase in TOPF-FSIQ (CR2 applied: $\beta=0.29$, $p_{CR2}=.0016$). A significant effect for group implies that relatives scored $z=0.42$ points lower than controls, holding all else constant (CR2 applied: $\beta= -0.64$, $p_{CR2}=.004$). The interaction indicated that relatives showed a greater increase in BNT scores over time compared to controls (CR2 applied: $B(SE)=0.253(0.124)$, $\beta=0.38$, $p_{CR2}=.044$).

EMMs show that the positive interaction effect for relatives did not eliminate the performance gap however. Controls performed significantly better at baseline (T1; EMM estimate(SE)=0.423(0.15), $p=.007$), and still better, but not significantly so, at T2 (EMM estimate(SE)=0.17(0.15), $p=.261$).

Table 24: Linear mixed effects model for confrontation naming (BNT) for relatives & controls over two timepoints.

Confrontation naming	B(SE)	df	t	β	p	Robust SE p_{CR2}^a
Step 1						
Fixed effects						
Constant	0.63 (0.2)	36.43	3.147	0.24	.003	.012*
Interval	-0.01 (0)	25.6	-2.754	-0.29	.011	.012*
Group	-0.55 (0.15)	46.87	-3.695	-0.83	<.001	<.001*
Timepoint (T1-T2)	0.14 (0.09)	72	1.532	0.21	.13	.155
Interaction (Group $\times$ Time)	0.25 (0.12)	72	2.075	0.38	.042	.044*
Step 2						
Fixed effects						
Constant	-1.27 (0.66)	44.45	-1.915	0.14	.062	.031*
Interval	-0.01 (0)	26.9	-3.475	-0.35	.002	.002*
Group	-0.42 (0.15)	52.49	-2.874	-0.64	.006	.007*
Timepoint (T1-T2)	0.14 (0.09)	72	1.532	0.21	.13	.155
Interaction (Group $\times$ Time)	0.25 (0.12)	72	2.075	0.38	.042	.044*
TOPF-FSIQ	0.02 (0.01)	46.21	2.98	0.29	.005	.002*
Random effects				SD		
ID within Family				0.394		
Family				0.155		
Residual				0.368		

NOTE: Table shows unadjusted coefficients. Age accounted for by z-scores. Education did not improve model above that of TOPF-FSIQ. ^a Robust p-values calculated using cluster-robust standard errors (CR2).

6.3.2.3.4 LMM: Semantic fluency (animals)

For semantic verbal fluency, model diagnostics indicated small violations of linearity and homogeneity of variance. Two high leverage points were removed for sensitivity analyses. Neither sensitivity analysis nor cluster-robust SEs changed the direction or significance of the outcomes, though slightly reduced the strength. There

were no significant fixed effects of group ($\beta=0.25$), only time and the interaction were statistically significant (**Table 25**). Scores improved by over one standard deviation ($B(SE)=1.06(0.19)$, $\beta=0.98$, $p<.001$) between timepoints, but unaffected fALS relatives improved much more modestly than controls, evidenced by the large significant negative interaction effect ($B(SE)= -0.88(0.25)$, $\beta= -0.81$, $p<.001$), decreasing semantic fluency z-scores by 0.88 points for every unit increase in the interaction of relatives over time.

EMMs showed that controls scored lower at T1 though not significantly so (T1 EMM estimate(SE): fALS relatives=0.06(0.17); controls= $-0.22(0.19)$, $p=.292$). EMM differences were significant at follow-up however, with controls improving and scoring significantly higher than fALS relatives (T2 EMM estimate(SE): relatives=0.24(0.17); controls=0.84(0.19), $p=.024$; **Table 25**).

Table 25: Linear mixed effects model for semantic verbal fluency (category: animals)

Semantic (Category) Fluency	B(SE)	df	t	β	p	Robust SE p_{CR2}^a
Fixed effects						
Constant	-1.6 (1.14)	31.25	-1.403	-0.4	.171	.09
TOPF-FSIQ	0.01 (0.01)	30.23	1.264	0.13	.216	.127
Group	0.27 (0.25)	44.95	1.088	0.25	.282	.179
Timepoint (T1-T2)	1.06 (0.19)	72	5.627	0.98	<.001	<.001*
Interaction (Group $\times$ Time)	-0.88 (0.25)	72	-3.499	-0.81	<.001	<.001*
Random effects				SD		
ID within Family				0.676		
Family				0.169		
Residual				0.754		

^a Robust p-values calculated using cluster-robust standard errors (CR2).

NOTE: Table shows unadjusted coefficients. Age accounted for by z-scores. Education did not improve model above that of TOPF-FSIQ.

6.3.2.3.5 LMM: RFI

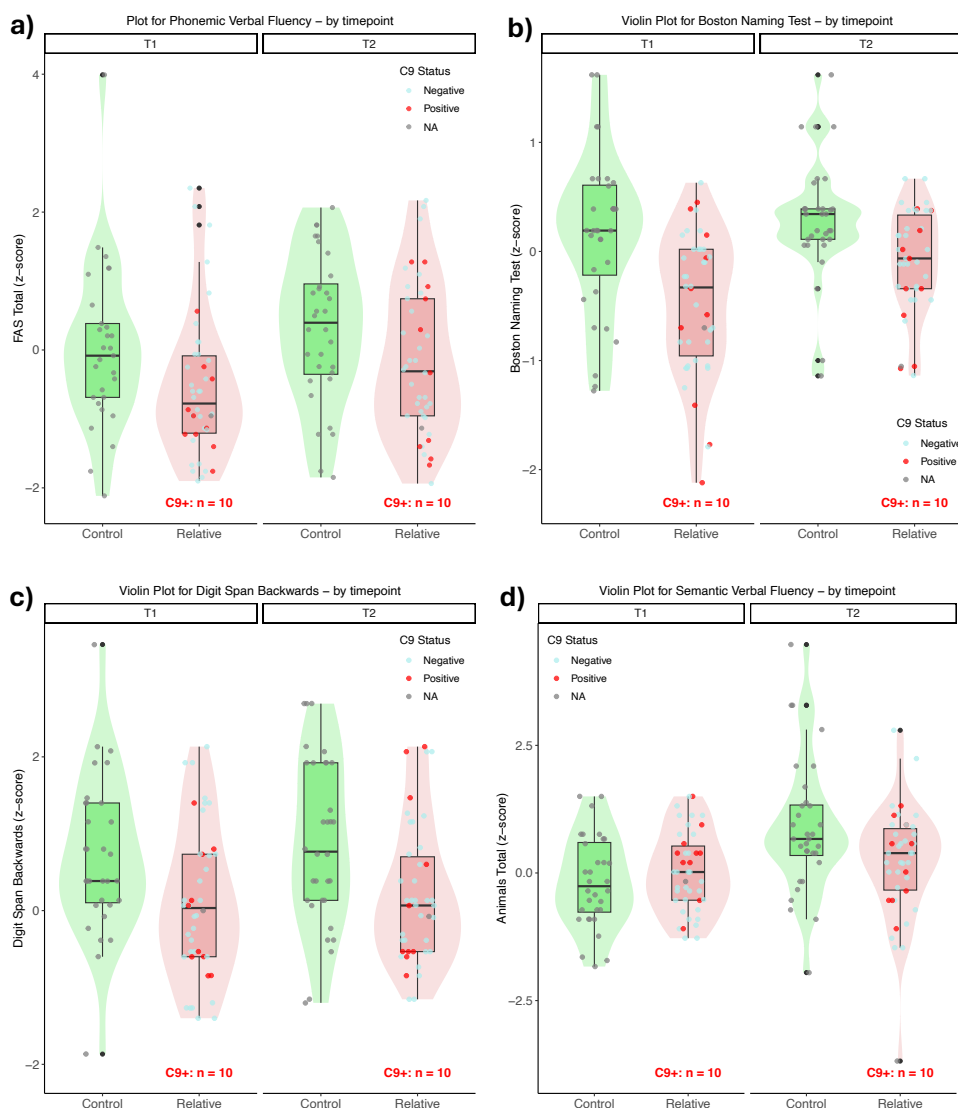
There was low clustering within family for RFI scores among relatives ($ICC=0.109$), indicating minimal between-family variability. A LRT for interval time was not significant ($p=.49$). Model diagnostics indicated no violations of assumptions.

The model revealed a significant interaction between group and timepoint (T2), suggesting that the change in RFI scores over time differed between relatives and controls ($B=1.02(0.31)$, $\beta=0.78$, $p=.002$; **Table 26**; **Figure 28**). There was also a main effect of timepoint, with a decline in scores at T2 ($B= -0.81(0.24)$, $\beta= -0.63$ $p=.001$). The main effect

of group showed that this effect was marginal ($p=.082$), suggesting the difference may be explained by the interaction term. TOPF-FSIQ was a significant predictor, with higher premorbid IQ associated with higher RFI scores ($\beta=0.23$, $p=.041$).

EMMs indicated that controls showed a reduction in performance at T2, while relatives improved. Despite this interaction effect, relatives still underperformed relative to controls at T1 (EMM = -0.44 vs. 0.15 , $p=.087$) but reached near-equivalence at T2 (EMM = 0.22 vs. -0.06 , $p=.216$).

Figure 27: Violin plots of the key cognitive variables of interest in the longitudinal cognitive endophenotype study.

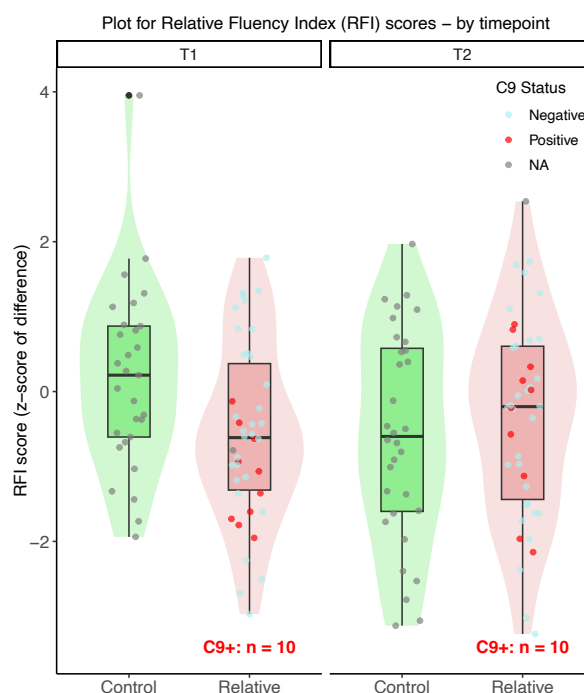


NOTE: a) FAS – phonemic verbal fluency, b) BNT – confrontation naming, c) DSB – working memory, d) semantic verbal fluency. Before controlling for TOPF-FSIQ, fALS relatives score significantly lower on each of the three candidate endophenotypes over time (a-c), showing less practice effects than control participants over time (T1-T2). Overlaid dot plot shows independence of *C9orf72*-status for all tasks.

Table 26: Linear mixed effects model for the Relative Fluency Index (RFI) for controls and relatives, over time, controlling for premorbid IQ estimates (TOPF-FSIQ).

RFI	B(SE)	df	t	β	p
Fixed effects					
Constant	-2.84 (1.49)	55.67	-1.90	-0.39	.062.
TOPF-FSIQ	0.03 (0.01)	54.39	2.10	0.23	.041*
Group	-0.60 (0.34)	56.48	-1.77	-0.46	.082.
Timepoint (T1-T2)	-0.81 (0.24)	70.86	-3.43	-0.63	.001*
Interaction (Group $\times$ Time)	1.02 (0.31)	69.77	3.27	0.78	.002*
Random effects					SD
ID within Family					0.757
Family					0.461
Residual					0.916

Figure 28: Violin plot for RFI z-scores for controls and fALS relatives over time.



6.3.3 Equivalency: TOST for temporal stability

Ranges and descriptive statistics for test change scores used to create the TOST upper and lower bounds are given in **Appendix 17**.

6.3.3.1 Paired t-test

The paired t-tests showed statistically significant improvements from T1 to T2 for the fALS relatives on the FAS, BNT and current IQ variables (all $p < .05$), with large effect sizes (FAS: Hedges $g = 0.42$; BNT: Hedges $g = 0.78$; **Table 27**). WASI-II FSIQ-2 was not

significant after correction for multiple comparisons however ($p_{\text{adj}}=.052$; **Table 27**). These improvements likely reflect practice effects. The DSB, semantic fluency and RFI did not demonstrate significant change over time.

6.3.3.2 TOST: upper- & lower-bounds

All assumptions of the TOST were met for each variable of interest. All tests were equivalent at the lower bounds before correction for multiple comparisons, indicating stability over time when controlling for practice effects (see **Figure 29**). Although they did not demonstrate change from baseline on the paired t-test, after FDR correction, digit span backwards and semantic fluency were no longer significant at the lower bounds (unadjusted $p=.04$ and $p=.034$ respectively; see **Table 27**), suggesting that for these tests, equivalence and stability over time is not supported. No test was equivalent overall after FDR correction ($p_{\text{adj}}>.05$ for at least one bound), indicating that change scores for the fALS relatives were not equivalent to those of the controls.

RFI change scores showed large variation within controls over time ($\Delta T2-T1$ Range= $-3.29-2.32$), thus creating wide bounds. There was also large variation at the individual level in unaffected fALS relatives, but displayed little change between T1 and T2 on the RFI at the group level ($\Delta M(\Delta SD)=0.2(1.25)$, $\Delta Md=0.02$; **Figure 28**). There was no significant difference on the paired t-test, and the lower-bound TOST was highly significant, suggesting scores were stable for fALS relatives ($t(41)=1.05$, $g(SE)=0.201(0.19)$, $p_{\text{adj}}=.302$). The mean difference for fALS relatives surpassed the control upper bound threshold however, meaning the equivalence test was not significant overall ($t(41)=2.22$, $p=.98$; **Table 27 & Figure 29**).

6.3.4 Effect of C9-status over time

The above analyses were repeated for unaffected fALS relatives only, with C9-status as the grouping factor, to investigate the effects of carriership on cognitive scores over time using linear mixed-effects models (LMMs). **Figure 30** displays extended battery variables by *C9orf72*-status over the two timepoints. Sub-group analyses of ECAS scores were also included for this cohort. Due to the relationship between ECAS scores, age and education, these were included as covariates in the ECAS models. Final models include random-effects structure of individual nested within family (1|family/id) to account for the hierarchical data structure. A LRT showed that inclusion of family as a random effect did not significantly improve model fit for any variable. It was nonetheless retained for consistency. **Appendix 17** shows the outcomes of the full analysis.

Table 27: Two one-sided t-test (TOST) for equivalence of the extended neuropsychological assessment over the two timepoints for the fALS relatives (n=42).

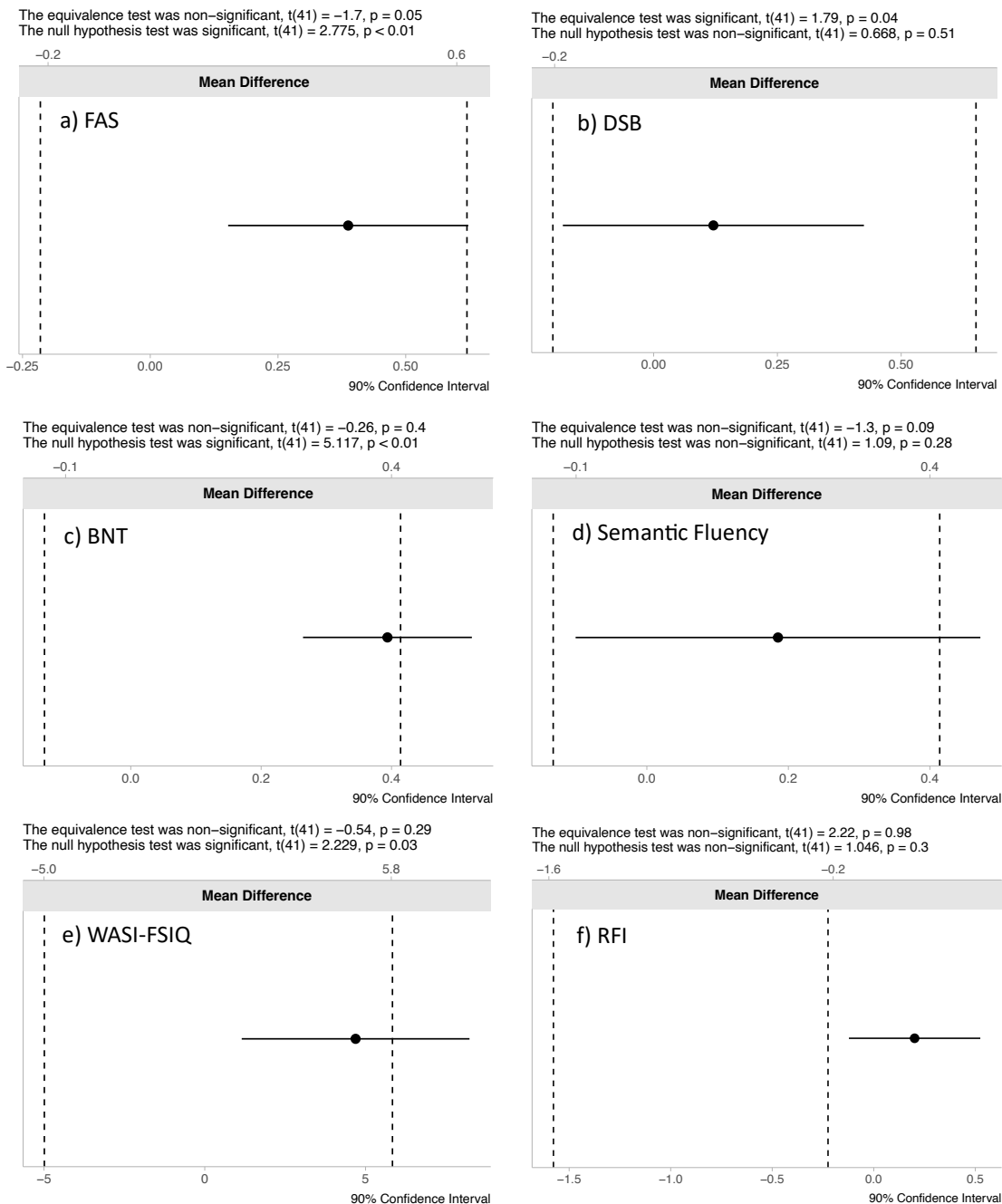
	t (df)	g (SE)	p	p.adj
FAS				
Paired t-test	2.775 (41)	0.420 (0.16)	.008	.02
TOST Lower	4.316 (41)		<.001	.002
TOST Upper	-1.665 (41)		.052	.074
DSB				
Paired t-test	0.668 (41)	0.101 (0.16)	.508	.508
TOST Lower	1.794 (41)		.04	.067
TOST Upper	-2.936 (41)		.003	.008
BNT				
Paired t-test	5.117 (41)	0.775 (0.18)	<.001	.003
TOST Lower	6.837 (41)		<.001	.002
TOST Upper	-0.262 (41)		.397	.397
Semantic fluency				
Paired t-test	1.090 (41)	0.165 (0.16)	.282	.353
TOST Lower	1.869 (41)		.034	.067
TOST Upper	-1.345 (41)		.093	.116
Current IQ (WASI-II FSIQ)				
Paired t-test	2.229 (41)	0.338 (0.16)	.031	.052
TOST Lower	4.599 (41)		<.001	.002
TOST Upper	-0.545 (41)		.294	.327
RFI score				
Paired t-test	1.046 (41)	0.201 (0.19)	.302	.302
TOST Lower	9.253 (41)		<.001	<.001
TOST Upper	2.218 (41)		.984	.984

NOTE: g = Hedges g (z-standardised) effect size estimate. Negative value in the t-tests for upper TOST limits indicate the observed mean difference is lower than the upper equivalence bound, and positive lower bound t-value indicates the observed mean is higher than the lower bound. Both tests being statistically significant indicates H_0 for the TOST can be rejected. p.adj = corrected for multiple comparisons using the Benjamini-Hochberg False Discovery Rate (FDR).

6.3.4.1 C9 LMM: Phonemic verbal fluency

There was moderate clustering within families for the FAS LMM (ICC=0.199). Only TOPF-FSIQ significantly predicted FAS scores (B(SE)=0.038(0.015), β = 0.38, p=.017). C9+ relatives scored lower than C9- relatives, but this was not statistically significant (B(SE)= -0.60(0.38), β = -0.56, p=.11). EMMs showed a narrowing of the group difference from T1 to T2, but differences were not significant at either timepoint. These findings suggest that FAS scores were modestly influenced by premorbid IQ, with a non-significant trend for lower scores in C9+ relatives; variance was well distributed across levels: family (SD=0.53), individual (SD=0.67), and residual (SD=0.64).

Figure 29: TOST results for the extended neuropsychological assessment.



NOTE: TOST with upper and lower bounds determined by Cohen's $d = 0.5$ of the change scores for control participants for each test. a) phonemic verbal fluency (FAS), b) working memory (Digit Span Backwards), c) confrontation naming (Boston Naming Test), d) semantic fluency (Animals category), e) current IQ (WASI-II FSIQ), and f) Relative Fluency Index (RFI).

6.3.4.2 C9 LMM: Digit span backwards

Clustering within families was low ($ICC=0.112$). The final model included nested random effects and CR2 robust SEs due to a mild violation of homoscedasticity (Breusch-Pagan $p=.015$). Only TOPF-FSIQ significantly predicted DSB scores ($B(SE)=0.039(0.008)$, $\beta=0.47$, $p_{CR2}<.001$). No effects were found for C9-status ($\beta=-0.07$, $p_{CR2}=.846$), timepoint ($\beta=0.05$, $p_{CR2}=.808$), or their interaction ($\beta=0.33$, $p_{CR2}=.521$). EMMs showed no significant group differences (C9+ vs C9-) at either timepoint (T1: $p=.846$; T2: $p=.450$). Variance was highest at the residual level ($SD=0.843$), with minimal family ($SD \approx 0$) and low individual-level variance ($SD=0.122$). These findings suggest that DSB performance was primarily influenced by premorbid IQ estimates, with no evidence of C9-status or timepoint effects, and most variability attributable model error (residuals).

6.3.4.3 C9 LMM: Confrontation naming

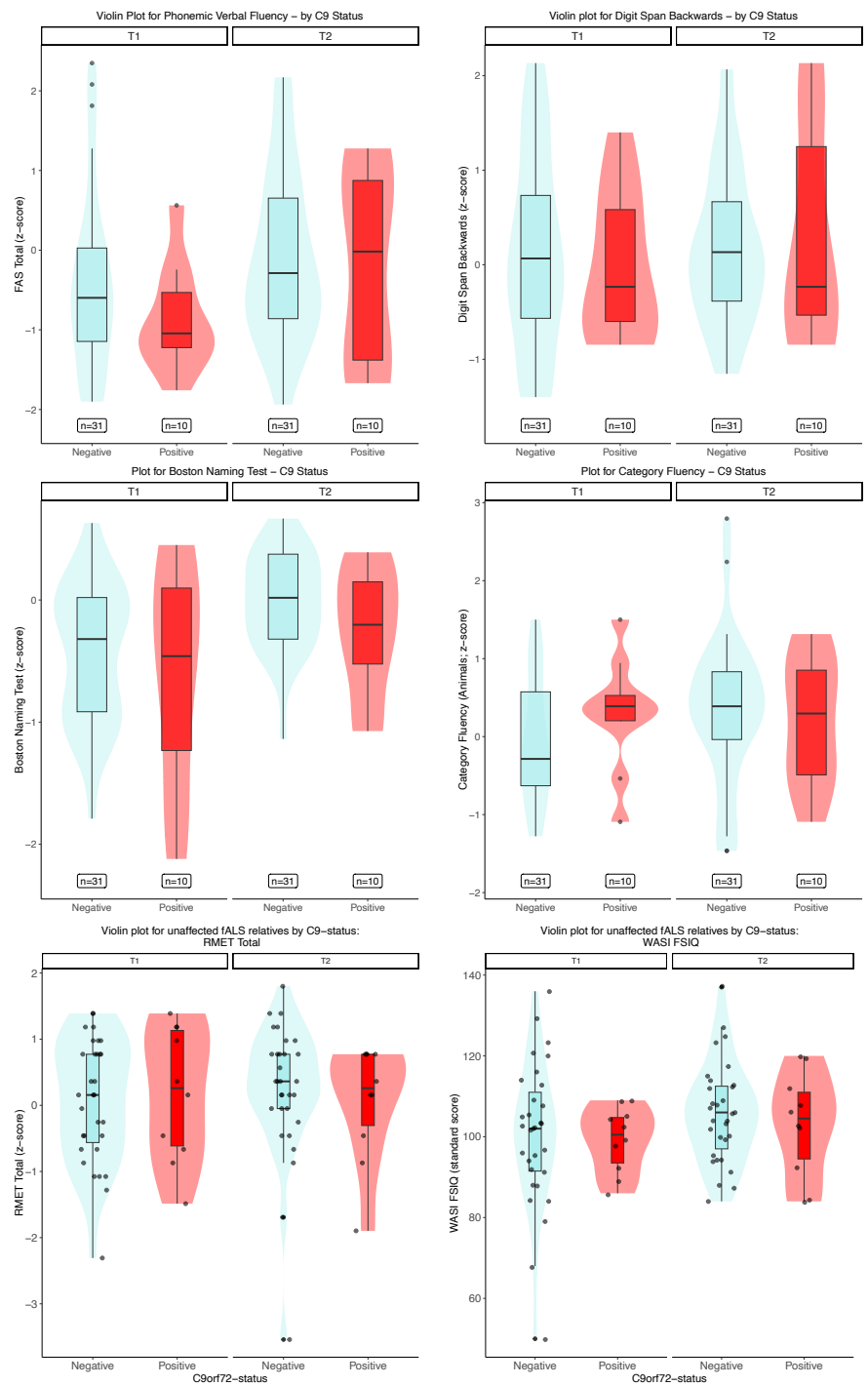
In the confrontation naming LMM for the effects of C9-status on BNT scores over time, family-level clustering was low ($ICC=0.062$). CR2 robust SEs facilitate model interpretation due to a mild violation of homoscedasticity (Breusch-Pagan test: $p<.001$).

Timepoint was the only significant predictor, with scores increasing by 0.432 points at T2 ($B(SE)=0.432(0.057)$, $\beta=0.72$, $p_{CR2}<.001$). A smaller effect of TOPF-FSIQ was also observed ($\beta=0.24$, $p_{CR2}=.029$). No effects were found for C9 status or the interaction ($\beta=-0.14$, $p_{CR2}=.773$). EMMs indicated group differences at T1 and T2 were non-significant. Most variance was at the individual level ($SD=0.415$), with small family-level ($SD=0.099$) and residual variance ($SD=0.35$). These findings suggest that confrontation naming scores improved over time regardless of C9 status. Performance was influenced by premorbid IQ, while family effects were negligible.

6.3.4.4 C9 LMM: Semantic fluency

Clustering within families was low ($ICC=0.065$) for the semantic fluency LMM. Model diagnostics did not indicate any violations of assumptions. Timepoint was the only significant predictor, with higher scores at T2 ($B(SE)=0.39(0.17)$, $\beta=0.46$, $p=.021$). No significant effects were observed for TOPF-FSIQ, C9-status, or their interaction ($\beta=-0.57$, $p=.156$). EMMs showed no significant group differences at either timepoint (both $p \geq .25$). These findings suggest that semantic fluency scores improved over time regardless of C9 status. Most variance was attributable to residual error ($SD=0.659$) and Individual-level differences ($SD=0.533$), with little contribution from family-level effects ($SD=0.152$).

Figure 30: Violin plots by C9-status for key neuropsychological variables within the longitudinal follow-up relatives sample.



NOTE: n=10 C9+ carriers completed the extended battery at longitudinal follow-up.

6.3.5 Longitudinal ECAS

Demographic data for the longitudinal ECAS group are show in **Table 28**. Distribution of ECAS scores are shown in **Figure 31a-c**.

Table 28: Baseline demographic data for the ECAS longitudinal sample.

	T1		T2	
	C9-	C9+	C9-	C9+
Baseline demographics				
n	33	17	33	17
Age	45.01 (15.3)	38.56 (10.7)	-	-
Sex (% Female)	42.4%	37.5%	-	-
Years Education	17.23 (3.1)	16.67 (3.2)	-	-
Testing interval (months)	-	-	60.1 (20.3)	52.25 (21.7)
ECAS Total	109.91(12.4)	114.1 (6.7)	116.06(9.3)	116.75 (8.0)
ECAS ALS-Specific	80.18(10.2)	84.56 (3.8)	85.38(7.3)	85.94 (7.2)
ECAS ALS-Non-Specific	29.68(3.1)	28.5 (5.0)	30.68(3.4)	30.81 (2.29)

NOTE: Participants are the same at T1 and T2. Scores provided are M(SD).

6.3.5.1 Measuring reliable change in unaffected fALS relatives (T1-T2)

Abnormality and meaningful decline on the ECAS over time was calculated for fALS relatives using published abnormality/RCI cut-offs (**Table 29**).^{162,163} At baseline, C9- relatives show higher rates of impairment on the ECAS (17.6% vs 0% on ALS-specific), but were similar at T2. Four relatives showed significant cognitive decline by RCI. Two were C9- (older age from the same family, declined on non-specific domain) and two C9+ (age 44 and 55 at T1). One C9+ relative declined on ALS-specific only, the other on both ALS-specific and ECAS Total. Only the latter participant showed abnormal scores and declined reliably at T2, the remainder either stayed within normal limits or did not decline on RCI.

Table 29: ECAS domain abnormality classifications for fALS relatives by C9orf72-status.

	T1		T2		RCI Decline
	C9-	C9+	C9-	C9+	n
ECAS Total	4 (11.8%)	0 (0.0%)	2 (5.9%)	1 (6.2%)	1 ^a
ECAS ALS-Specific	6 (17.6%)	0 (0.0%)	2 (5.9%)	1 (6.2%)	2 ^a
ECAS ALS-Non-Specific	3 (8.8%)	1 (6.2%)	1 (2.9%)	1 (6.2%)	2 ^b

NOTE: RCI = Reliable Change Index. C9+/C9-=C9orf72-positive/-negative.

^a C9+ fALS relatives. n=1 declined on ALS-specific only & n=1 on ALS-specific & ECAS Total.

^b n=2 C9- fALS relatives from the same family & of older age (78 & 80 years old at T2).

6.3.5.2 C9 LMM: ECAS

LMMs for the effects of C9-status on ECAS scores over time include relatives with available genotype data only (n=50). Timepoint also includes ECAS version, which was ECAS B for all participants at T2. Models were constructed as follows:

$$\text{'ECAS domains'} \sim \text{'Age'} + \text{'Education'} + \text{'C9-status*Timepoint'} + (1 \mid \text{family/id})$$

Random-effects of family were predictive in isolation (indicated by ICC values), but the variability explained at this level is captured by the individual-level random intercept. Sensitivity analyses following violations of assumptions due to influential cases were performed for ECAS Total and ALS-Specific. The direction or strength of the outcomes did not change with the sensitivity analyses, and so all participants were included and CR2 SE-derived p-values are reported.

Overall, education and age were significant predictors, as well as timepoint/ECAS version in all models, so that higher education, lower age, and second timepoint were independently associated with higher ECAS scores ($p_{\text{CR2}} < .05$). Improvements in ECAS scores were seen across the three domains (see ALS-Specific model in **Table 30**).

Fixed effect for timepoint on ECAS Total showed improvement of 6.96 points from ECAS A (T1) to B (T2; CR2 SEs applied: $B(\text{SE})=6.96(1.57)$, $p_{\text{CR2}} < .001$). Fixed effects of C9-status and the interaction (C9xtime) were not significant for ECAS total or ALS-non-specific domains ($p > .05$). ALS-specific ECAS domain scores however were significantly higher for C9-carriers after cluster-robust SEs were applied ($B(\text{SE})=5.7(2.12)$, $p_{\text{CR2}} = .012$). The interaction of C9-status and timepoint was also significant, but in the opposite direction, attenuating the effect, so that ALS-specific scores were 5.48 points worse at T2 for C9-carriers (Robust SEs applied: $B(\text{SE}) = -5.48(2.21)$, $p_{\text{CR2}} = .019$). Random effects

indicated low family-level variability for ECAS Total score (SD=1.85) relative to individual differences (SD=5.13) and residual variance (SD=5.89).

Unaffected fALS C9+ relatives show higher scores on the ECAS, but only at baseline. EMMs find low ALS-specific scores for C9- fALS relatives at T1, which improved significantly at T2/ECAS B, likely as a regression to the mean. This would explain the negative interaction effect in the C9+ group (see **Figure 31**).

Table 30: Linear mixed effects model for ECAS ALS-specific scores in fALS relatives only by C9orf72 status.

ECAS ALS-Specific	B(SE)	df	t	β	p	Robust SE p_{CR2}^a
Fixed effects						
Constant	63.21 (5.24)	57.06	12.06	-0.46	<.001	<.001
Age	-0.1 (0.06)	47.76	-1.72	-0.17	.092	.141
Education	1.23 (0.24)	58.74	5.23	0.48	<.001	<.001
C9 Status (C9+)	5.7 (2.05)	81.92	2.78	0.69	.007	.012
Timepoint (T1-T2)	5.68 (1.31)	53.5	4.36	0.68	<.001	<.001
Interaction (C9+$\times$Time)	-5.48 (2.25)	48.67	-2.44	-0.66	.019	.019
Random effects				SD		
ID within Family					4.03	
Family					0.00	
Residual					5.24	

^a Calculated with CR2 cluster robust standard errors

NOTE: Reported are the original unadjusted coefficients.

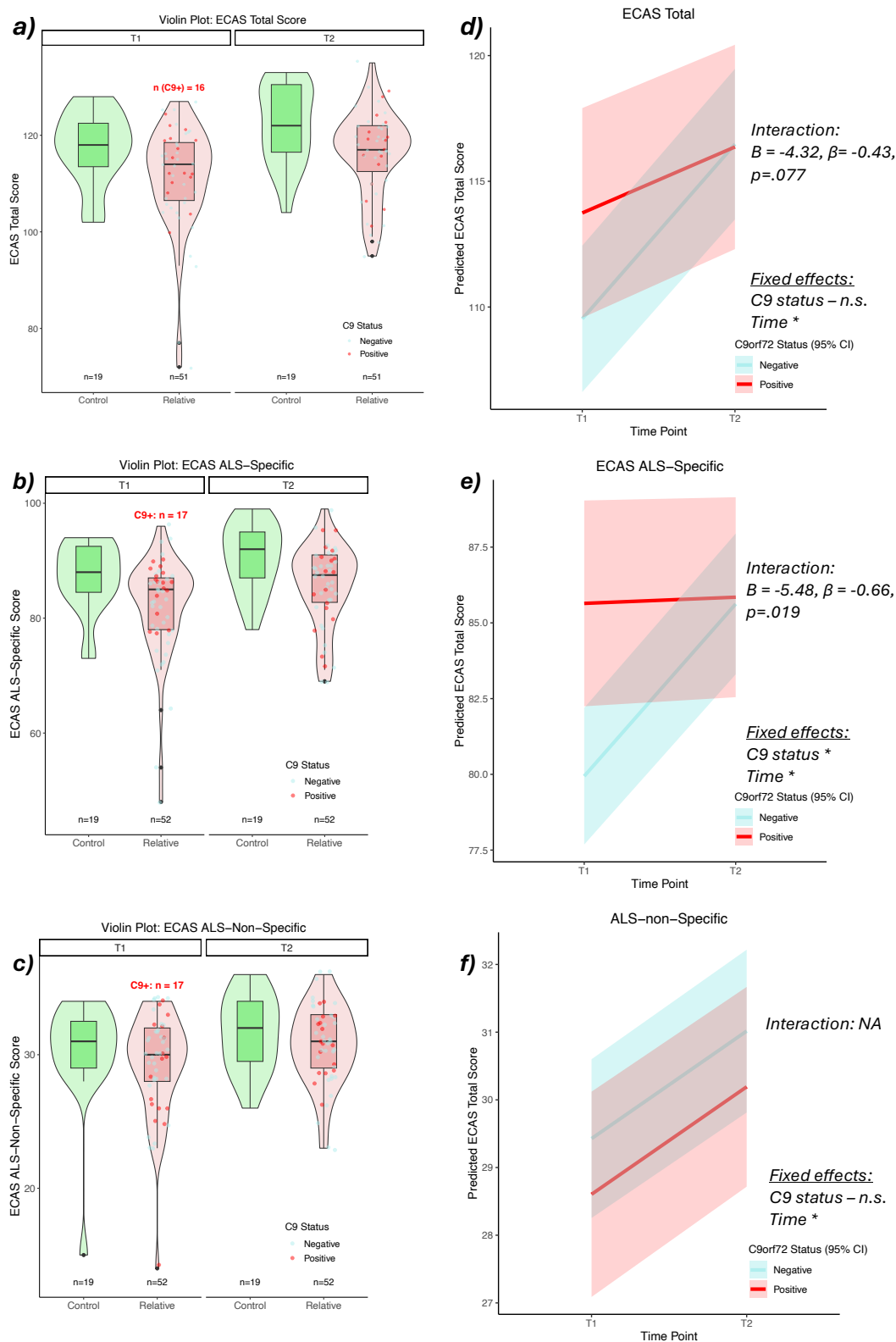


Figure 31: Longitudinal ECAS in unaffected fALS relatives.

a-c) show violin plots of ECAS Total, ALS-specific and ALS-non-specific scores between groups over time. C9-status indicated by red/blue dots. **d-f)** shows interaction plot of model-predicted marginal mean ECAS scores over time for fALS relatives only, by C9 status holding all other predictors constant.

6.4 Discussion:

These studies investigated the stability of putative cognitive endophenotypes with particular focus on phonemic verbal fluency, working memory, and confrontation naming and the effects of *C9orf72* genetic status on cognitive performance over time in asymptomatic relatives of fALS patients. A new outcome (RFI) for isolating the differential effect of ALS-specific cognitive impairment (semantic vs phonemic verbal fluency discrepancy) is tested in this unaffected fALS relatives sample.

6.4.1 *RFI: Standardised fluency discrepancy score*

This chapter tested the use of a standardised difference score, which aims to target the selective higher-order executive impairment of phonemic fluency in ALSci.¹¹⁸ The RFI factors out task-based effects (speed, time-pressure, error monitoring, etc.) within an individual controlling for age and education, giving a measure of differential deficit between phonemic and semantic verbal fluency performance, if present.^{525,526} In theory, it gives a purer measure of differentially affected higher order executive networks in ALS.^{118,141} A similar differential score has demonstrated utility in differentiating dementia subtypes.¹⁴⁸ Different in principal from the VFI, the RFI indirectly controls for verbal motor dysfunction while providing a measure-specific function through comparison of standardised performances on differentially affected tasks.

This study showed increased error from use of the RFI, and so effect sizes in the replications study decreased somewhat for the Phase 1 sample compared to FAS scores alone. However, the effect in the Phase 2 sample demonstrated a larger discrepancy in fluency scores, replicating the Phase 1 effects despite FAS scores alone failing to replicate.

6.4.1.1 *RFI performance over time*

The RFI did not perform as expected in the longitudinal study. Contrary to the hypothesis, there were significant and opposite changes in relative fluency performance. RFI scores increased over time for unaffected fALS relatives, but decreased in the control group, the relative fluency gap closing slightly and increasing respectively. Examining the tasks individually shows that this effect was caused by a significant improvement in semantic fluency scores but phonemic fluency scores stayed relatively stable in fALS relatives. The large and significant main effects for group, and the group×time interaction in the semantic fluency mixed-effects model indicates differential practice effects for

controls, explaining this effect. The control group shows larger gains on semantic fluency scores over time compared to phonemic fluency, and compared to unaffected fALS relatives, who improved similarly on both tasks.

If unequal practice effects alter the relationship between the fluency tasks, the utility of the RFI therefore may be limited to baseline assessments. Given the large testing intervals here, strategy-based practice effects are low to negligible, but familiarity in the easier task (semantic fluency) may have boosted performance.¹⁵¹ Nonetheless, the RFI shows utility here at Phase 1, Phase 2 and at baseline in the longitudinal studies, supporting **Hypothesis 7.1**.

6.4.2 Replication study: baseline comparisons of Phases 1 & 2

The Phase 2 replication study further supports the presence of cognitive endophenotypic traits in an independent, well-matched case-control cohort of unaffected fALS relatives (**Hypothesis 4.1**). z-test comparisons of effect sizes and random-effects meta-analysis with heterogeneity analysis replicated the profile of unaffected fALS relatives as characterised by subtly decreased scores on executive (working memory; DSB) and language (confrontation naming; BNT) with medium effect sizes ($d > .55$). Mixed results arise from the FAS test (phonemic verbal fluency), which demonstrated the strongest effects as a putative endophenotype in Phase 1.

6.4.2.1 FAS test: Failure to replicate?

Unaffected fALS relatives performed worse than controls on the FAS task at baseline in Phase 1 and Phase 2. Despite the consistent direction, group differences on the task were not significant in Phase 2, which does not support a replication of the effect. Results from the longitudinal follow-up do provide support for the FAS test as a cognitive endophenotype however. Large effects demonstrated by the FAS task in Phase 1 were maintained over time in the longitudinal study ($p = .026$), albeit attenuated by premorbid IQ estimates ($\beta = -0.29$, $p_{CR2} = .180$).

Conflicting evidence for the FAS task implies there are differences between the Phase 1 and Phase 2 cohorts. Both samples are demographically similar with only minor improvements to matching for age and premorbid and current IQ estimates in Phase 2. In any case, the large effect sizes for phonemic verbal fluency at Phase 1 were significant after controlling for age, education and full-scale IQ estimates. These factors are therefore unlikely to have caused failure to replicate here. There were different examiners for Phase 1 and 2, which may have introduced scoring-bias. However, protocols were shared, and

interrater reliability studies were performed, ensuring consistency. Furthermore, the effects of all primary test remained in the longitudinal study, indicating reliability of scoring for the Phase 1 baseline sample across to Phase 2.

RFI scores provide strong evidence for replication of the effect. The differential deficit method yielded a medium effect size ($d_{\text{pooled}}=0.488$) which was highly statistically significant by the random-effects meta-analysis and z-test statistic across both baseline replication phases. This index score theoretically isolates dorsolateral-prefrontal function within the superior medial fronto-striatal circuit, accentuating the selective impairment of this higher-order executive task, which is differentially affected in ALS.^{117,118} Improved matching may explain improvements to semantic fluency at an equivalent level to phonemic fluency between the Phase 1 and Phase 2 samples, as both of these scores are highly related to general intellectual functioning.^{138,663,664} Further support for this is the increased effect size in a positive direction for the Digit Span Forwards task of simple attention, which also correlates moderately with IQ.⁶⁶⁵

Significant group differences on memory tasks (RAVLT, RCFT) were accounted for by IQ in Phase 1.²¹⁰ Scores on these tasks demonstrated increases in Phase 2 so that fALS relatives, proportional to relative increases in IQ (TOPF-FSIQ, WASI-II) which was closer to that of control participants. Although these effects do not appear to replicate in Phase 2 prospective sample meta-analysis, showing decreased effect sizes on the z-test, this is a reflection of improved sample-matching. This all supports the replication of the effects demonstrated in Phase 1, with tighter controls of intellectual function and age.

6.4.3 Longitudinal MND Family study

Longitudinal follow-up with a sample of fALS relatives showed cognitive trait differences are largely stable over time relative to control participants. Increases were evident on most tasks between timepoints, despite the long intervals. In every case, TOPF-FSIQ was a significant predictor in the final LMMs, often capturing the effects demonstrated by group (relatives vs controls) in Step 1 of the hierarchical models. Nonetheless, the temporal stability of the three primary neuropsychological measures, and others within the battery, is largely supported.

6.4.3.1 Trait temporal stability

Results of the TOST indicate that fALS relatives did not deteriorate on tests over time compared to controls. Significant results at the lower-bounds for all six cognitive variables of interest supports the hypothesis that unaffected fALS relatives cognitive

characteristics and performance were stable over time. DSB and semantic fluency did not survive correction for multiple comparisons however, despite the DSB meeting criteria for full significance before correction. This is reflective of improvements over time which were not as large as controls, as opposed to a decline over time. Hierarchical linear mixed-effects regression models investigated this relationship further.

6.4.3.2 Modelling variability over time

The series of models found fALS relatives scored lower on all cognitive tests of interest, before the addition of TOPF-FSIQ in Step 2. Strongest evidence was again for confrontation naming (BNT), which was the only variable for which fixed-effects remained in the final model. While temporal stability is supported, as relatives did not demonstrate decline over time, it indicates that the effects are explained by group differences in premorbid IQ. Interaction effects, suggestive of divergent trends across groups, were only significant for confrontation naming, indicating fALS relatives improved more than controls, though still scored lower overall, and semantic fluency, which showed controls experienced more significant gains over time.

Here, although sample size was small ($n_{FB}=10$), *C9orf72*-status was not associated with confrontation naming performance at baseline or in the longitudinal study, though was significantly lower in fALS relatives compared to controls. Reductions in confrontation are common in pwALS^{114,141,532,666}, and have been demonstrated in asymptomatic at-risk *MAPT* carriers from FTD kindreds.⁶⁶⁷ Lexical retrieval, captured by the BNT here, has high potential as a candidate cognitive endophenotype in ALS in addition to phonemic verbal fluency.

6.4.3.2.1 Family effects

Cognitive scores showed some shared variance between family members. The effects were very small however. Nonetheless, it is important to explore these effects in family-based designs. In the context of C9-ALS, where genetic penetrance varies between kindreds^{75,77}, subtle familial patterns of cognitive traits may provide insight into underlying genetic liability. Recent findings of genetic discordance in ALS kindreds⁸⁰ further supports the hypothesis that risk from alternative genetic mechanisms are present in kindreds, not explained by C9-status alone. Endophenotypes therefore remain of critical importance to uncover unknown oligogenic contributors.

6.4.3.2.2 *Controlling for TOPF-FSIQ*

Longitudinal models controlled for the effects of TOPF-FSIQ, which was a highly significant predictor of nearly all cognitive variables in the presented analyses. In Step 1 of all hierarchical models, there were large effects of group. These may be over-corrected when additionally controlling for premorbid IQ in Step 2, particularly as groups were matched for educational attainment and z-scores were adjusted for age and/or education. In this context, lower TOPF-FSIQ scores may not solely reflect sampling bias or demographic mismatch but could index a true group-level difference associated with endophenotypic traits. Dyslexia and self-endorsed reading difficulties were prevalent in ALS family histories, and three participants were excluded due to dyslexia diagnoses. Reading ability and developmental dyslexia both show a polygenic basis with relatively high heritability.⁶⁶⁸⁻⁶⁷¹ Rather than a confound, reduced TOPF-FSIQ performance may itself be suggestive of a cognitive trait, relevant to familial genetic liability in ALS. In this case, statistically controlling for TOPF-FSIQ obscures the true magnitude of the effects.

6.4.4 *Longitudinal cognition in asymptomatic C9orf72-carriers*

C9orf72-status was not associated with significant group effects on the ECAS or extended cognitive test battery in the current study sample. There is a flat progression of scores in the C9+ group for the ALS-specific domain in which they perform similarly at both timepoints.

6.4.4.1 *Evidencing reliable change*

The RCI findings of the current study are concordant with those of McHutchison et al. (2024)¹⁷³ who report age-related decline in older pwALS on the ALS-non-specific domain, but only C9orf72-positive pwALS showed significant declines on ALS-specific domains. Numbers are small in the current study, but RCI thresholds used are valid for detection of longitudinal change over time in pwALS, and provide strong evidence of a meaningful clinical decline.¹⁶² This participant did not have longitudinal FB data available for comparison.

6.4.5 *Study-specific limitations*

General biases in recruitment are addressed in the discussion. Unique to these studies are the multiple phases of attrition and potential bias of the captured sample displayed in the recruitment flow charts (**Figure 21** & **Figure 23**). Many participants did not

elect to complete the extended neuropsychological assessment at baseline, only completing the ECAS. There was a high proportion of C9+ relatives in this sample (52% of those who declined; 100% were from C9-kindreds). A number of these participants, included here in the ECAS study, do not have longitudinal FB data, reducing the power of the longitudinal stability study to interrogate sub-group effects of *C9orf72*. These participants provided longitudinal ECAS data however (n=17), demonstrating no effects. C9 status was not available in this sample to replicate the findings with relation to independence from *C9orf72*, or to provide increased power to the Phase 1 C9-subgroup analyses.

In addition to gene-status, representativeness of the longitudinal sample was affected by self-selection bias, shown in part by the lower education and ECAS scores of relatives who declined longitudinal assessment in Phase 2. Despite confrontation naming being the most robust effect for temporal stability in the captured sample, decliners also showed significantly lower confrontation naming performance, which would have increased the strength of the effect. Practice effects are not expected to significantly impact cognitive test scores over intervals as long as this (Md=3.83 years)⁵³⁷, although improvement across groups and timepoints is consistent across tests.

6.4.5.1 ECAS study limitations

Unbalanced group sizes were also present, with only 19 controls in the ECAS study. This was to ensure that testing procedures were the same for all participants, and necessitated the exclusion of 12 controls who completed ECAS B T1. This was also a factor related to the discrepancy in the interval time, where recent controls were more likely to have completed ECAS A T1. The time between testing was significantly longer in the fALS relatives group on the ECAS (not extended neuropsychological assessment), which may have biased test scores. However, the average interval in the controls was 28 months (2.33 years) in the ECAS study, and alternate forms of the ECAS were used (A at T1 and B at T2). Testing interval did not improve LMM predictions, indicating no significant effect.

6.5 Conclusions:

RFI performance, indicative of a differential network dysfunction, and stability over time in the longitudinal study together provides support for replication of phonemic verbal fluency as a candidate cognitive endophenotype in unaffected fALS relatives. This is demonstrated alongside smaller effects of decreased working memory (DSB) and confrontation naming (BNT). The current studies interrogate group differences with improved control sample matching for age, and systematic control for premorbid IQ estimates, which support the findings of Costello²¹⁰ for an effect of general IQ on memory function. In addition, temporal stability is demonstrated, which supports these cognitive tasks as meeting Gottesman and Gould's criteria for an endophenotype.

Unaffected fALS relatives perform worse than controls on the ECAS consistently over time. *C9orf72*-status had no bearing on cognitive performance for the ECAS. All scores demonstrated stability over time consistent with control levels of practice effects.

Together these finding suggest trait differences are independent of disease state and stable over a 4 year period and so may be the product of alternative genetic factors conveying an increased susceptibility to ALS in extended kindreds. This is in line with the multistep hypothesis of ALS where selective medial-frontal and prefrontal network vulnerabilities are likely present in cases with increased genetic liability.

7 Chapter 7:

Results: A HD-Cognition cross-sectional baseline study

7.1 Introduction

Social cognitive dysfunction is key cognitive domain in the classification of mild and major neurocognitive dysfunction under the DSM-V.⁶⁷² A recent review from the *SIGNATURE* initiative found only 5% of respondents reported routinely assessing social cognition in major and mild NCDs.³⁶⁶ Assessment of social cognition is not yet standard for PwHD.³¹⁰

Studies of PwHD which do assess social cognition typically only measure affect recognition.^{358,359,410,673,674} More recent studies also include other domains and Theory of Mind (ToM)^{365,675} ToM ability has high potential as early cognitive biomarker of cognitive dysfunction in HD^{356,470} but the results from existing studies are contradictory, with some finding significant changes in premanifest PwHD groups³⁵⁴, where other studies do not.⁶⁷⁶

There are only two longitudinal studies of ToM function in HD identified by Escuerdo-Cabarcas et al.³⁶⁴, and the development of social cognitive features of HD in the premanifest phase is under explored. Rather than a single domain-specific deficit, social cognition impairments may reflect disruption across a system of interrelated processes.⁶⁷⁷ It is therefore pertinent to explore function across multiple social cognitive domains in the neuropsychological assessment of HD.

7.1.1 Assessments of social cognition

The Reading the Mind in the Eyes Test (RMET), Eckman 60 Faces and the Faux Pas test are the most commonly used tasks for the assessment of ToM.^{130,358,364,366} The RMET in particular is widely used, with a number of short-forms created.^{549,678,679} The RMET, and other ToM tasks, have shown sensitivity to early changes in manHD but the majority find

no group differences for premanifest PwHD compared to matched controls.^{375,470,680,681} An imaging study found premanifest RMET performance was associated with connectivity in the amygdala.⁶⁸² Other studies in PwHD have reported associations between RMET performance and functional imaging markers, which in turn correlate with estimated motor symptom onset and age.^{470,682-684} Sample sizes are generally small in premanifest studies, likely contributing to the inconsistent findings.^{358,364}

Psychometric reviews of the validity of the RMET have reported concerns about item construction and linguistic complexity.^{679,685} Others have shown RMET performance is related to executive function in ALS.¹²⁹ Nonetheless, the RMET has demonstrated sensitivity to deficits in affective ToM and affect recognition across a range of clinical populations, including autism spectrum⁵⁴⁷, bvFTD⁶⁸⁵, ALS¹²⁹ and HD.³⁵⁴

Consensus that impairments of visual facial affect recognition is established in HD, but there is less evidence for aural or vocal affect recognition and other social perception deficits, such as sarcasm detection and as body language.^{358,359} The ACS Social Perception Battery (ACS-SP) has been validated in various clinical samples^{551,552,686-691}, but has not yet been used in HD. The ACS-SP improves on the ecological validity of older affect recognition and social cognitive assessments, and has standardised and demographically-adjusted normative data. This enhances diagnostic sensitivity and helps delineate patterns of social cognitive strengths and weaknesses.

7.1.1.1 Task impurity for social cognitive & the executive functions

It has been argued that deficits in social cognitive function in neurodegenerative disorders are the result of global factors, attributable to executive functioning and decline in general intellectual functioning. Existing studies of social cognition in HD often fail to control for the additional cognitive demands in tasks of social cognition. There is a relationship between executive function and RMET scores in ALSci⁵⁴⁸, but no such association has been reported in HD.³⁶⁵ Inconsistent results in the premanifest stage, may be due to issues with construct validity in the RMET, including an overreliance on linguistic ability, executive search difficulties and impulsivity.⁶⁹²

In addition to global factors affecting social cognitive test scores, most studies of cognition in HD report executive dysfunction but do not account for speed of processing in timed tasks.^{228,345,392,494} Verbal fluency tasks are commonly employed in early HD, but both semantic and phonemic verbal fluency have been found to load more on processing speed than executive or linguistic ability however.⁶⁹³ In HD, unlike the selective impairment in ALSci, performance on both phonemic and semantic verbal fluency are

equivalently impaired^{140,376,694}, possibly explained by this overlap with speed of processing.

7.1.2 Chapter aims

This chapter addresses the cross-sectional aspects of **Aims 5, 6** and **7**, and corresponding **Hypotheses (5.1-5.3, 6.1, 6.4, 7.2)** investigating social cognitive function as a sensitive marker of early Huntington's disease. It will examine group differences and associations to disease progression estimates, as well as task validity and the influence of verbal IQ on performance. The analysis will also explore the confounding effects of processing speed in interpreting executive function outcomes in PwHD and evaluates the discriminant validity of the RFI (**7.2**).

7.2 Methods

This study is a baseline analysis of the HD-Cognition study and follows the methodology outlined in **Chapter 4**.

7.2.1 Participants

Recruitment for the HD-Cognition study was performed by convenience sampling, outlined in the methods section. Participants fell into three groups at baseline: manHD (n=14), preHD (n=38) and control (n=40) participants. Three participants were excluded from the baseline analysis for meeting exclusion criteria. The premanifest and control groups were matched for demographic variables age, sex, education; **Table 31**). For all PwHD participants with available CAG-repeat data, CAP100 is used across the disease span to as an estimate of CAG-related disease progression.³¹⁵ Premanifest PwHD additionally were categorised as far (>10 years), near (≤ 10 years) and past (< 0) estimated motor-symptom onset.^{71,392}

7.2.2 Materials

Full neuropsychological assessment, including questionnaire data, are analysed in this baseline study of HD-Cognition, with a focus on tasks of social cognition and executive function. Primary social cognitive tests of interest are the RMET and ACS-SP subtasks, which include Affect Naming, Prosody-Face Matching and Prosody-Pair Matching.⁵⁵⁰⁻⁵⁵² Additional key variables include outcomes of the SDMT, CWIT, verbal fluency tasks, and the digit span forwards and backwards. Contrast scores from the CWIT Inhibition subtest isolate the effects of cognitive inhibition from general processing speed. VFI scores are assessed for utility against the uncorrected z-scored fluency tasks.

7.2.2.1 Neuropsychiatric & behavioural assessment

Apathy and apathy sub-types in the HD-Cognition study are discussed in a subsequent chapter (**Chapter 9**). The current chapter reports self-reported and companion-/carer-reported questionnaire data. Tests and domains included are shown in **Table 40** which also shows self-report measures of Quality of life (QoL; EuroQoL), Fatigue (Multidimensional Fatigue Symptom Inventory; MFSI) and Disability (WHO-Disability Assessment Schedule; WHODAS). Recent recommendations from the HSG call for the use of the WHO Disability Assessment Schedule (WHODAS) as a measure of functional status in HD alongside the UHDRS.³¹⁰

7.2.2.2 *Functional status*

The UHDRS is the standard measure of motor function, functional capacity and independence in HD. Scores are obtained by structured interview with the participant (Manifest and Premanifest groups). Totals for each domain are given in **Table 31**.

7.2.3 *Statistics*

Group-level differences were assessed using a one-way analysis of covariance (ANCOVA). Models were fitted for each cognitive outcome as the dependent variable (DV), with group (Manifest, Premanifest, Control) as a between-subjects factor (independent variable) and TOPF-FSIQ as a covariate.

Each model was specified as:

$$\text{'Cognitive score'} \sim \text{'group'} + \text{'TOPF-FSIQ'}$$

Each model evaluated the effect of group after adjusting for premorbid IQ using a statistical framework (Type III SS) that isolates each effect regardless of the order entered. Then, the F-statistic and p-value for group were extracted to assess whether the groups differed significantly on the cognitive variable. Given the expectation that the Manifest group will show reduced performance across all cognitive domains relative to Controls, and the primary focus of this thesis is on presymptomatic cognitive function, a priori planned contrasts specifically assess differences between the Premanifest and Control groups for each statistically significant ANCOVA.

Estimated marginal means (EMMs) were calculated for planned contrasts between premanifest and control groups applying a custom contrast vector (control – premanifest). Partial eta-squared (η_p^2) estimated the size of the main effect of group, controlling the covariate. All contrast p-values were corrected using the Benjamini-Hochberg procedure to control for the FDR across multiple comparisons. Both unadjusted and adjusted p-values are shown.

For questionnaire data, group comparisons were conducted using one-way ANCOVAs, or Kruskal-Wallis H-tests where assumptions of normality were violated. All participant reported outcome measures are reported as raw scores, and are not standardised by age or education. Therefore age is controlled for in all parametric analyses (ANCOVA).

The model used was:

$$\text{'Questionnaire score'} \sim \text{'group'} + \text{'age'}$$

As with cognitive variables, planned contrasts using EMMs were computed following a significant ANCOVA, between control and premanifest groups. For non-parametric data, pairwise Wilcoxon rank-sum tests were conducted.

7.2.3.1 Social cognitive function

The RMET does not have published clinical cut-offs for abnormality.⁵⁴⁸ To examine rates of impairment on the RMET, a cut-off for abnormality was set at $z = -1.65$, which corresponds to the 5th percentile. To evaluate the discriminative utility of three of the main social cognitive tasks in differentiating between manHD participants and healthy controls, a receiver operating characteristic (ROC) curve analyses was performed. Optimal thresholds based on the AUC are applied to premanifest PwHD to classify abnormality. ROC results are interpreted alongside planned group comparisons and effect size estimates to determine the most sensitive test for detecting social cognitive deficits in the premanifest phase. These thresholds will also be applied to premanifest participant scores to inspect rates of impairment.

7.2.3.1.1 Factor analysis for social cognition subtests

Simple correlational analyses between scores on the RMET and Affect Naming and Prosody-Face Matching ACS-SP subtests were conducted to explore the associations between tasks. To assess the dimensional structure and construct validity of the social cognition battery, an exploratory factor analysis (EFA) was subsequently performed to determine whether the tests captured distinct social cognitive constructs. Maximum likelihood extraction with oblimin rotation was used to allow for potential correlation between latent constructs. The number of factors to retain was determined using parallel analysis with 100 Monte Carlo simulations and inspection of the associated scree plot. Analyses were conducted using the 'psych' package in R.⁶⁹⁵

7.2.3.2 Hierarchical regression for RMET & vocabulary

Performance on the RMET depends upon a comprehension of often, low-frequency descriptions of complex affective and cognitive states. Hierarchical linear regression was performed to assess the extent to which group differences in RMET scores were explained by verbal ability, measured by the WASI-II vocabulary subtest, whilst also

controlling for premorbid IQ estimates. The vocabulary subtest of the WASI-II is a measure of crystallised verbal intelligence. Scores are calculated from age-stratified normative data to standard T-scores. For conversion of WASI-II subtest T-scores and Standard Scores to z-scores, the following formula was applied:

Equation 12: z-score conversion from a) T-scores & b) WASI-II FSIQ-2 Standard Scores.

$$\text{a)} \quad z = \frac{T - 50}{10}$$

where, 50 is the mean (μ) and 10 is the standard deviation (σ) of the T-score distribution.

$$\text{b)} \quad z = \frac{FSIQ - 100}{15}$$

where, 100 is the mean (μ) and 15 is the standard deviation (σ) of the normal distribution for IQ.

To differentiate the dependency of tests on verbal and general ability, the ACS-SP subtests, which demand less of language and verbal ability, are also assessed. Standardised beta values (β) from linear regression models are compared between the RMET and Affect Naming task—which uses high-frequency words as descriptors of fundamental emotions, i.e.: Happy, Sad, Afraid, Surprise, Disgust, Neutral—and the RMET and Prosody-Face Matching—which is not reliant on language. Baseline hierarchical regression model includes TOPF (Step 1), with additional steps to the final model (Step 3) indicated here:

‘social cognition subtest’ ~ ‘TOPF-FSIQ’ (1) + ‘WASI-II Vocab’(2) + ‘group’ (3)

7.3 Results

Demographic data for the whole sample are given in **Table 31**, and for premanifest groups in **Table 32**. Although manifest participants were older and had slightly lower education, groups did not differ significantly (both $p > .08$). There were a higher proportion of females in control and premanifest groups compared to the manifest group ($p = .04$). Expectedly, they had significantly worse functional scores and higher measures of disease burden compared to premanifest participants ($p < .05$; **Table 31**). Groups did not differ on CAG repeat length ($p > .05$).

Baseline scores and statistics for each neuropsychological test of interest by group are given in **Table 39**.

The groups differed on TOPF-FSIQ estimates with a large effect size ($\eta^2 = 0.2$, $p < .001$), EMMs showed that all groups differed significantly from the others ($p < .05$). It was therefore used as a covariate in ANCOVA analyses to follow.

7.3.1 Intellectual functioning

Current intellectual function was assessed by the WASI-II FSIQ-2, which includes subtests of Vocabulary and Matrix Reasoning. One-way ANCOVAs, controlling for premorbid IQ estimates, found significant differences between groups on overall FSIQ-2 score ($F(2,84) = 42.37$, $p < .001$), and also on the sub scales (Vocabulary: $F(2,85) = 38.61$, $p < .001$; Matrix Reasoning: $F(2,84) = 13.98$, $p < .001$; **Figure 32a-c**). Planned comparisons also indicated that premanifest participants scored lower on the vocabulary subtest, but this did not reach significance after correction for multiple comparison (EMM estimate = 0.53(0.18), $p_{\text{adj}} = .059$). There were no other significant differences on the WASI-II ($p > .05$).

7.3.2 Social cognitive function in HD

7.3.2.1 The RMET

Figure 33b illustrates the rates of impairment on the RMET across the groups. 69.23% of manifest participants fell in the abnormal range by clinical cut-offs ($z = -1.65$), with only 7.89% and 2.62% in the premanifest and control groups respectively. Fisher's exact test indicated these were significant differences ($p < .001$), but premanifest and control groups did not differ ($p = .6$).

An ANCOVA was performed on the continuous RMET z-scores, which was significant across groups with a large effect ($\eta_p^2=0.41$, $p < .001$), group explaining 41% of the variance in RMET scores, holding TOPF-FSIQ constant. Estimated marginal means indicated that there were no differences between control and premanifest groups (estimate=0.19(0.24), $d=0.186$, $p_{adj}=.643$; **Figure 33a**). An interaction model was used to assess whether the relationship between TOPF-FSIQ and RMET performance varied across groups. The interaction term was not significant ($p=.577$), supporting the assumption of homogeneity of regression slopes (ANCOVA assumption).

Table 31: Demographic variables for HD-Cognition sample at baseline (T1).

	Controls	Premanifest	Manifest	df	F/ χ^2	p
n	40	38	14	-	-	-
Age M(SD)	45.68 (12.5)	44.64 (11.57)	53.25 (13.65)	2,89	2.6	.08
Sex (% Female)	65%	66.7%	31.2%	2	7	.04
Years Education M(SD)	17.11 (2.8)	16.61 (2.85)	15.25 (2.84)	2,89	1.85	.164
HD only variables					t/χ^2	p^f
CAG # available (n) ^b	-	30	11			
CAG # M(SD)	-	41.9 (2.55)	43.82 (3.92)	10	1	.2
CAG # (range)	-	39-52	39-53			
CAP100 ^{b,c}	-	82.13 (21.81)	108.44 (15.75)	16	4	.002
Disease Burden ^{b,d}	-	272.75 (91.64)	395.41 (94.23)	12	3	.007
ttdx (years) ^{b,e}	-	10.37 (13.33)	-3.64 (9.44)	-	-	
UHDRS-Function Md(IQR)	-	25 (0)	19 (8.75)	-	72	<.001
UHDRS-Function Range	-	19 – 25	8 – 25	-	-	
UHDRS-TFC Md(IQR)	-	13 (0)	9.5 (5.25)	-	94	<.001
UHDRS-TFC Range	-	5 – 13	3 – 13	-	-	
UHDRS-Ind. Md(IQR)	-	100 (0)	80 (25)	-	82	<.001
UHDRS-Ind. Range	-	80 – 100	55 – 100	-	-	

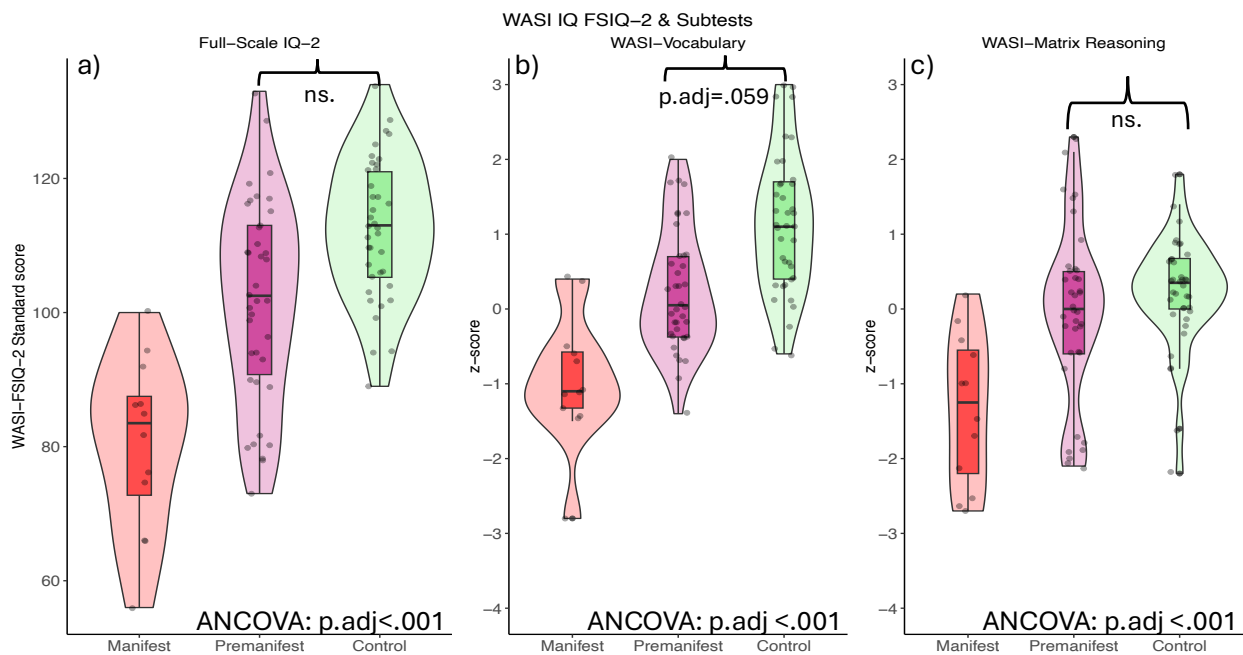
NOTE: UHDRS-Ind. = UHDRS Independence scale; TFC = Total Functional Capacity; ^a Not significant in post-hoc tests or after correction for multiple comparisons; ^b 6 manHD and 2 preHD cases did not have CAG repeat numbers available to calculate Disease burden and CAP100 scores; ^c A CAP100 score of 100 is the normalised CAGxAGE product estimated age of motor symptom disease onset based on Warner and Sampaio (2016)³¹⁵; ^d Disease Burden calculated according to Penney et al. (1997).³¹²; ^e ttdx = time to predicted diagnosis by Langbehn et al.(2004)⁷¹; ^f compares HD groups only.

Table 32: Demographic, CAG-related and Functional scores for Premanifest group stratified by proximity to estimated diagnosis based on Langbehn et al.

	Premanifest – Proximity to estimated dx		
	Far	Near	Past
CAG # available (n) ^b	13	12	7
Age M (SD)	37.23 (8.32)	45.58 (10.11)	56.14 (10.3)
Sex (% Female)	53.80%	66.70%	85.70%
Years Education M (SD)	17.15 (2)	16.33 (3.11)	15.86 (3.8)
CAG # M (SD)	40.62 (1.33)	43 (3.19)	42.57 (1.7)
CAG # (range)	39-43	40-52	41-46
CAP100 ^{b,c}	62.54 (13.23)	90.19 (6.56)	110.5 (10.4)
Disease Burden ^{b,d}	187.31 (49.35)	314.79 (40.02)	384.07 (38.6)
Time to predicted onset ^e (years)	22.62 (9.05)	4.5 (2.84)	-5.71 (5.6)
UHDRS-Function Md (IQR)	25 (0)	25 (0)	25 (0)
UHDRS-Function Range	23-25	22-25	24-25
UHDRS-TFC Md (IQR)	13 (0)	13 (0)	13 (0)
UHDRS-TFC Range	12-13	12-13	12-13
UHDRS-Ind. Md (IQR)	100 (0)	100 (0)	100 (0)
UHDRS-Ind. Range	100-100	100-100	100-100

NOTE: UHDRS-Ind. = UHDRS Independence scale; TFC = Total Functional Capacity; ^a Not significant in post-hoc tests or after correction for multiple comparisons; ^b 6 manHD and 2 preHD cases did not have CAG repeat numbers available to calculate Disease burden and CAP100 scores; ^c A CAP100 score of 100 is the normalised CAGxAGE product estimated age of motor symptom disease onset based on Warner and Sampaio (2016)³¹⁵; ^d Disease Burden calculated according to Penney et al. (1997)³¹²; ^e time to predicted diagnosis by Langbehn et al.(2004)⁷¹.

Figure 32: Group differences on a) WASI-II FSIQ-2 estimate of current IQ, b) WASI-II Vocabulary subtest, and c) WASI-II Matrix Reasoning subtest.

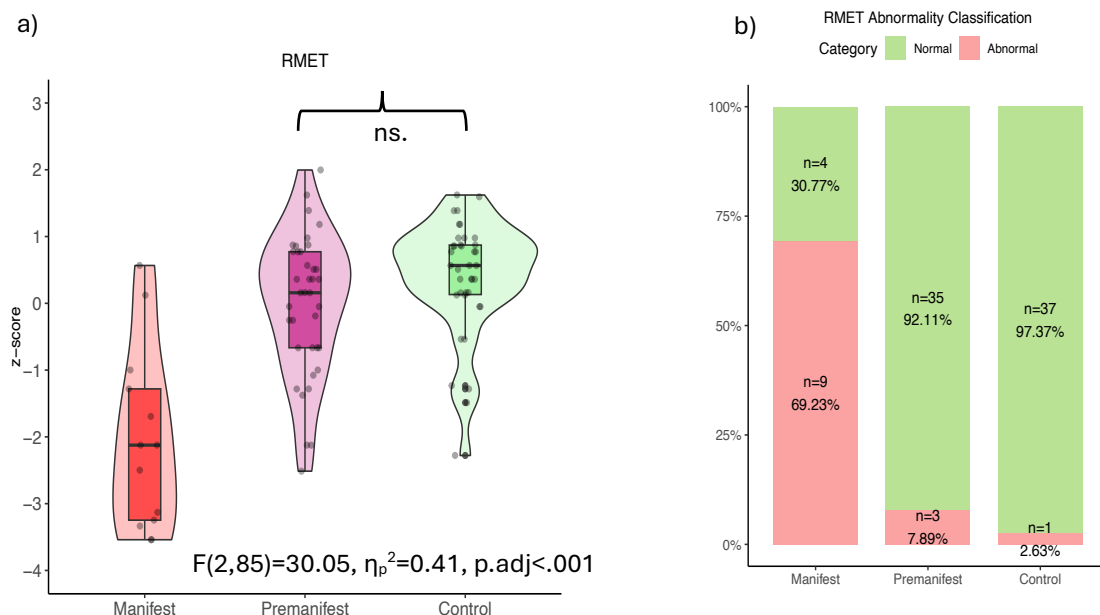


7.3.2.2 The ACS-SP

ANCOVA analysis for the three domains of the ACS-SP found significant group differences on all three, for Affect Naming ($F(2,74)=30.43$, $\eta_p^2=0.51$, $p.\text{adj}<.001$), Prosody Face Matching ($F(2,71)=24.02$, $\eta_p^2=0.4$, $p.\text{adj}<.001$) and the more complex subtask, Prosody Pair Total score ($F(2,57)=22.72$, $\eta_p^2=0.47$, $p.\text{adj}<.001$), each demonstrating very large effect sizes. After controlling for premorbid IQ (TOPF-FSIQ; $\eta_p^2=0.14$), 51% ($\eta_p^2=0.51$) of the remaining variance in Affect Naming is explained by group differences (e.g., manifest vs. premanifest vs. controls). Planned contrasts showed that these effects were driven by manHD participants, as there were no significant differences, before or after correction for multiple comparisons, between control and premanifest groups on any of the three subtests ($p/p.\text{adj}>.05$; see **Table 39**).

Effect sizes were small for both Affect Naming ($d=0.06$) and Prosody-Face Matching ($d=0.102$), but were slightly larger, although still small on the Prosody-Pair subtest ($d=0.275$). The overall combined Social Perception Scale also reflected this pattern ($F(2,57)=29.96$, $\eta_p^2=0.51$, $p.\text{adj}<.001$; control vs premanifest planned contrasts: estimate(SE)=0.56(0.7), $d=0.234$, $p.\text{adj}=.62$), but numbers are reduced.

Figure 33: RMET scores in the HD-Cognition Baseline sample: a) violin plot of group performance; b) rates of impairment.



NOTE: cut-off for impairment set as $z < -1.65$ (from published control sample data⁵⁴⁸).

7.3.3 Proximity to diagnosis & social cognition in premanifest HD

Sub-group analyses examined the association between cognitive function and time to estimated diagnosis in premanifest PwHD, controlling for premorbid IQ.

7.3.3.1 Social cognition: RMET & CAP100

In the premanifest group only, CAP100 score (available n=32) was a significant predictor of RMET performance in a linear regression model ($F(1,30)=6.47$, $R^2=0.18$, $p=.016$). CAP100 scores explained 18% of the variance and were negatively associated with RMET scores.

Comparing groups based on their proximity to diagnosis (far vs near vs past⁷¹), a one-way ANOVA revealed a significant difference in RMET scores ($F(2,26)=4.79$, $\eta^2=0.27$, $p=.017$), with a large effect size. Taken together, these results suggest that proximity to estimated onset is associated with differences in RMET performance reflecting CAG-related prediagnostic decline (**Figure 34**).

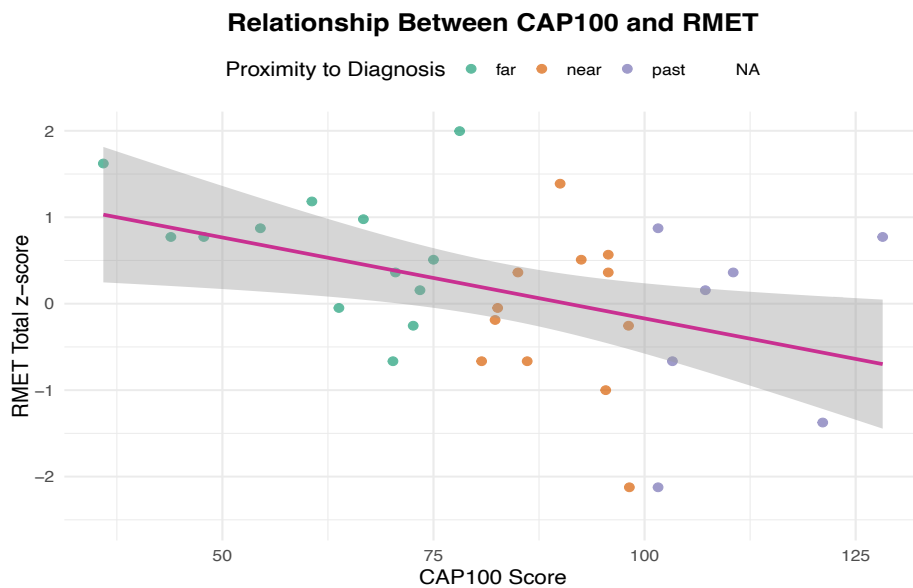


Figure 34: Scatter plots for RMET z-scores in relation to CAP100 scores in preHD. Dot-colour indicates proximity to diagnosis by Langbehn et al.'s model.

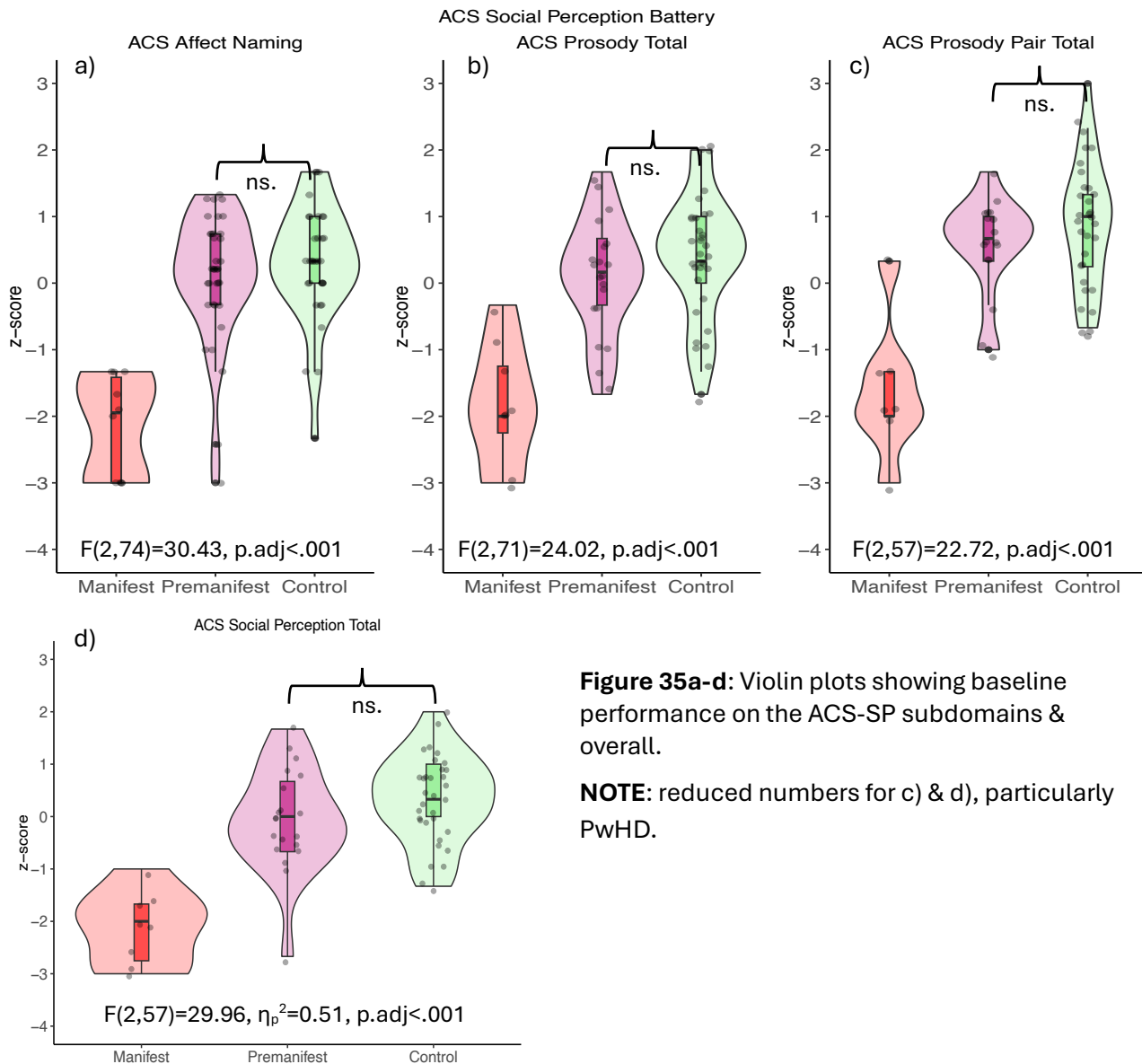


Figure 35a-d: Violin plots showing baseline performance on the ACS-SP subdomains & overall.

NOTE: reduced numbers for c) & d), particularly PwHD.

7.3.3.2 Social cognition: ACS-SP & CAP100

CAP100 score was not a significant predictor of Affect Naming performance in the premanifest group ($R^2=0.06$, $p=.192$), but was for Prosody face-matching ($R^2=0.27$, $p=.004$). Two outliers caused a violation of the assumption of normality of the residuals for the Affect Naming model ($p=.014$). Cook's distance was <1 , and so they were not considered highly influential. Given the robustness of linear models to mild violations of normality under these conditions, the model was retained.

Comparing these groups based on their time to predicted diagnosis⁷¹, one-way ANOVA were significant for Prosody face-matching, with a large effect size ($\eta^2=0.27$, $p=.017$). Levene's test showed a violation of the assumption of homogeneity of variances ($p=.008$) for the Affect naming subtest, and so Welch's F-test was performed, which did

not show evidence that the null hypothesis should be rejected ($\eta^2=0.30$, $p=.2$). Both tests have large effect sizes (27% & 30% of variance explained), which may indicate reduced power to detect significant effects in these sub-groups. **Figure 36** illustrates this effect.

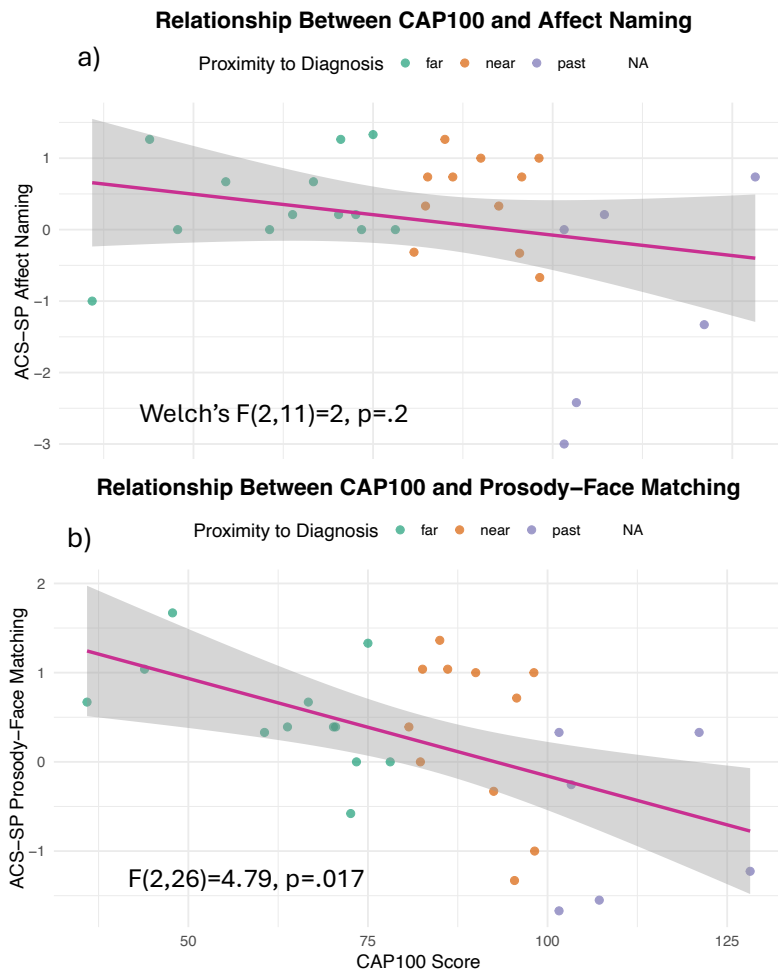


Figure 36: Scatter plots for the first two ACS-SP subtest:

a) Affect Naming and **b)** Prosody-Face Matching, in relation to CAP100 scores in the premanifest group. Dot-colour indicates proximity to diagnosis by Langbehn et al.'s model.

7.3.4 Thresholds for impairment on social cognitive tasks for manifest HD

All three social cognitive tasks used in the ROC analysis demonstrated strong discrimination between manHD and control participants (**Figure 37**). The ACS-SP Affect Naming subtest exhibited the highest diagnostic accuracy with an AUC of 0.973, achieving a sensitivity of 90.9% and perfect specificity (100%) at an optimal threshold of $z=-1.0$. The ACS-SP Prosody-Face Matching subtest also demonstrated excellent performance (AUC=0.96), with 75.8% sensitivity and 100% specificity at a threshold of $z=-0.165$. The RMET yielded a slightly lower but excellent AUC of 0.923, with 89.5% sensitivity and 84.6%

specificity at a threshold of $z = -0.768$. Optimal cut-offs and classification metrics were also reported (**Table 33**). Estimates should be interpreted with caution given the modest sample size and potential for overfitting ($n_{\text{manifest}}=14$).

DeLong's test indicated no statistically significant differences in classification between the Affect Naming and Prosody subtests ($z=0.30$, $p=.80$, 95% CI of AUC difference: $[-0.06, 0.08]$). This suggests that both tests are equivalently useful for distinguishing manifest PwHD from controls in this sample.

Table 33: Outcomes from ROC analysis and sensitivity / specificity at optimal threshold for the three social cognitive test domains.

Test	AUC	Sensitivity	Specificity	Optimal Threshold
RMET (roc1)	0.923	0.895	0.846	-0.768
Affect Naming (roc2)	0.973	0.909	1	-1
Prosody-Face Matching (roc3)	0.96	0.758	1	-0.165

7.3.4.1 Applying thresholds to premanifest HD

The optimal threshold cut-offs for classification between manifest and control groups in each subtest were applied to the premanifest group (see **Table 33**). Expectedly, as this cut-off is less stringent, rates are higher in the premanifest group than for the 5th percentile clinical cut-off applied in **Figure 33b**. At these thresholds, although the premanifest group have proportionally higher rates of 'impaired' performance, they are only marginally higher than controls (**Figure 38**).

Figure 37: ROC Curves for Social Cognition Tests. Controls vs ManHD group classification.

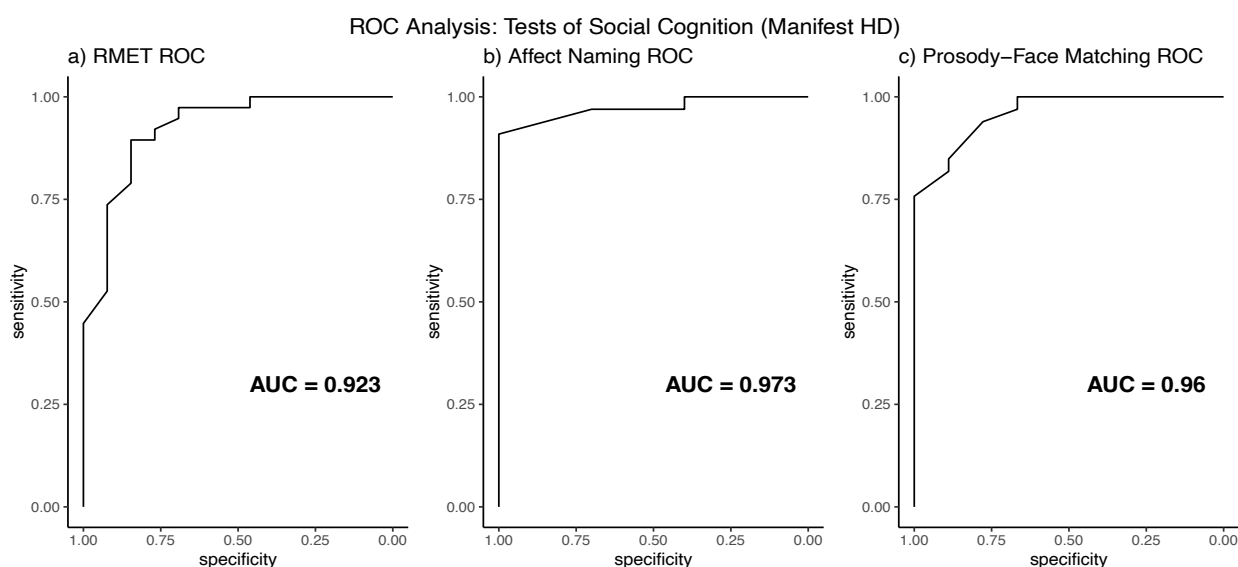
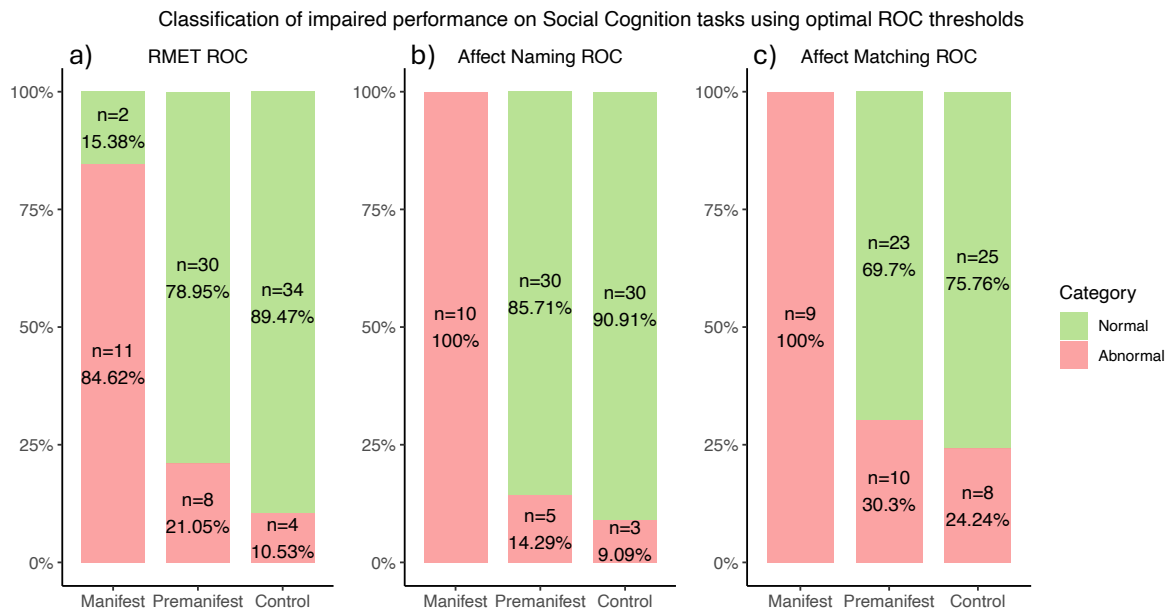


Figure 38: Social Cognition impairment rates under the ROC curve optimal threshold cut-offs.

NOTE: a) RMET, b) ACS-SP Affect Naming, and c) ACS-SP Prosody-Face Matching.



7.3.5 Assessing social cognition

7.3.5.1 Construct validity

Pearson product moment correlations (r) for parametric data were used to assess the strength of the relationship between social cognition subtests. p -values were adjusted for multiple comparisons. All three tasks were highly correlated (see **Table 34**). The RMET was as highly related to the ACS-SP subtests as they were to each other. Performance on the RMET was strongly correlated with Affect Naming ($r=0.70$, 95%CI:[0.56,0.80], $p<.001$) and with Prosody-Face Matching ($r=0.64$, 95%CI:[0.48,0.75], $p<.001$). Affect Naming and Prosody-Face Matching were also significantly associated ($r=0.62$, 95%CI:[0.45,0.74], $p<.001$). These results suggest moderate-to-strong convergence among tasks, supporting the construct validity of the RMET as an affect recognition task.

Table 34: Pearson's r correlation matrix of social cognitive subtests.

Pearson r	RMET	ACS: Naming	ACS: Prosody
RMET	1	p<.001	p<.001
ACS: Naming	0.696	1	p<.001
ACS: Prosody	0.636	0.615	1

NOTE: shown in white cells are Pearson's r, indicating the strength of the effect, and in grey are the p-values indicating statistical significance.

A factor analysis was then conducted on the three social cognition tasks, RMET, Affect Naming, and Prosody-Face Matching, to examine their dimensional structure. Parallel analysis, based on inspection of the scree plot indicated a one-factor solution, with no evidence supporting additional factors. A one-factor model explained 65.1% of the variance, with all tasks loading strongly: RMET ($\lambda=0.85$), Affect Naming ($\lambda=0.82$), and Prosody-Face Matching ($\lambda=0.75$). The two-factor model failed to improve model fit or interpretability, with the second factor explaining just 1.2% of the variance. This suggests that all three tasks reflect a single underlying construct, consistent with shared mechanisms of affect recognition and social cognition. But does not preclude the possibility that additional variance may be attributable to more specific ToM functions not captured by this factor analysis.

7.3.5.2 Hierarchical regression: contribution verbal ability to social cognitive task performance

To examine whether verbal ability contributed significantly to social cognitive task performance, hierarchical linear regression models were constructed for RMET, Affect Naming, and Prosody-Face Matching outcomes. The WASI-II Vocabulary subtest was included as a measure of crystallised verbal intelligence, while premorbid intellectual functioning (TOPF-FSIQ) was controlled for in Step 1 of all models as described in **section 7.2.3.2**. These analyses are visualised in **Figure 39**.

7.3.5.2.1 RMET & verbal ability

The baseline model explained 21.0% of the variance in RMET performance ($R^2=0.21$), with TOPF-FSIQ emerging as a significant predictor ($\beta=0.46$, $p<.001$; see **Table 35**). In Step 2, the addition of Vocabulary resulted in a statistically significant improvement in model fit ($\Delta R^2=0.105$, $p<.001$), explaining an additional 10.5% of the variance in RMET scores ($R^2=.315$). Vocabulary was a strong independent predictor

($\beta=0.43$, $p<.001$), and the predictive strength of TOPF-FSIQ was attenuated and no longer statistically significant ($\beta=0.18$, $p=.145$), indicating shared variance between premorbid and crystallised verbal ability. In Step 3, group was added to the model, further improving fit ($\Delta R^2=0.127$, $p<.001$), and the full model explained 44.2% of the variance in RMET performance ($R^2=.442$). Vocabulary remained a positive predictor, although its effect size was reduced and no longer significant ($\beta=0.22$, $p=.086$). Group differences remained for the manifest group in the final model, scoring significantly lower than controls ($\beta= -1.18$, $p<.001$). Premanifest and control groups did not differ ($\beta=0.01$, $p=.947$). For every unit increase in Vocabulary z-score, RMET performance increased by 0.25 SD, holding all else constant. Interaction effects between group and WASI-II Vocabulary scores did not significantly improve model fit ($p > .05$), suggesting that the association between vocabulary and RMET performance was consistent across groups.

7.3.5.2.2 ACS-SP: Affect naming & verbal ability

In the Affect Naming model (**Table 36**), the baseline model (Step 1) explained 9.4% of the variance in Affect Naming performance ($R^2=.094$). TOPF-FSIQ was as a significant predictor ($\beta=0.31$, $p=.007$). In Step 2, the addition of WASI-II Vocabulary significantly improved model fit ($\Delta R^2=.170$, $p<.001$), accounting for 26.4% of the variance in Affect Naming performance. Vocabulary was a strong independent predictor ($\beta=0.52$, $p<.001$), but TOPF-FSIQ was no longer significant ($\beta= -0.02$, $p=.898$). In Step 3, the addition of group yielded another large improvement in model fit ($\Delta R^2=.178$, $p<.001$). Vocabulary remained a significant predictor ($\beta=0.34$, $p=.009$). Group differences were evident between controls and manifest PwHD ($\beta= -1.41$, $p<.001$), but not premanifest PwHD ($\beta=0.10$, $p=.619$). The final model explained 44.2% of the variance in Affect Naming performance ($R^2=.442$). For every unit increase in Vocabulary z-score, Affect Naming performance increased by 0.39SD, holding all else constant.

Residuals in the final Affect Naming model deviated from normality, according to the Shapiro-Wilk test ($W=.90$, $p=.001$). However, given the robustness of linear regression to mild departures from normality and the known sensitivity of the Shapiro-Wilk test in samples over $n=30$, the model was retained. No other violations of assumptions were detected.

Table 35: Hierarchical linear regression: RMET vs WASI-II Vocabulary

RMET	(Δ)R ²	B (SE)	β	p
Step 1	0.21			
Constant		-5.19 (1.07)	0	<.001
TOPF-FSIQ		0.05 (0.01)	0.46	<.001
Step 2	0.105			<.001
Constant		-2.3 (1.29)	0	.077
TOPF-FSIQ		0.02 (0.01)	0.18	.145
WASI-II: Vocab		0.48 (0.13)	0.43	<.001
Step 3	0.127			<.001
Constant		-2.04 (1.22)	0.16	.099
TOPF-FSIQ		0.02 (0.01)	0.18	.105
WASI-II: Vocab		0.25 (0.14)	0.22	.086
Group: Premanifest		0.02 (0.25)	0.01	.947
Group: Manifest		-1.5 (0.42)	-1.18	<.001

7.3.5.2.3 ACS-SP: Prosody-face matching & verbal ability

For the Prosody-Face Matching model (**Table 37**), Step 1 explained 9.9% of the variance in performance ($R^2=.099$, adjusted $R^2=.088$), with TOPF-FSIQ emerging as a significant predictor ($\beta=0.31$, $p=.006$). In Step 2, Vocabulary improved model fit modestly ($\Delta R^2=.061$, $p=.026$). Vocabulary was a statistically significant predictor at this step ($\beta=0.32$, $p=.026$), though TOPF-FSIQ was no longer significant ($\beta=0.12$, $p=.412$). This model accounted for only 16.0% of the variance in task performance at Step 2. In Step 3, group was added and yielded a substantial improvement in model fit ($\Delta R^2=.219$, $p<.001$), with the full model explaining 37.9% of the variance ($R^2=.379$). Vocabulary was no longer a significant predictor ($\beta=0.08$, $p=.55$), and TOPF-FSIQ remained non-significant ($\beta=0.15$, $p=.22$). Yet again, the manifest group performed significantly worse than controls ($\beta= -1.66$, $p<.001$), while no significant differences were observed between premanifest and control groups ($\beta= -0.04$, $p=.87$). For every unit increase in vocabulary z-score, Prosody performance increased a negligible 0.09SD, which was not statistically meaningful when group was included.

7.3.5.3 Hierarchical regression: summary

Across all three tasks, the overall models at each step were statistically significant ($p<.01$), and model comparisons using ANOVA confirmed that the addition of variables at each step improved fit ($p<.05$). WASI-II Vocabulary subtest was the strongest independent predictor of performance on the Affect Naming task, accounting for premorbid IQ and

group ($\beta=0.34$, $p=.009$). In contrast, Vocabulary was not a significant predictor in the final models for RMET ($\beta=0.22$, $p=.086$) or Prosody-Face Matching ($\beta=0.08$, $p=.55$).

Table 36: Hierarchical linear regression: Affect Naming vs WASI-II Vocabulary

Affect Naming	(Δ)R²	B (SE)	β	p
Step 1	0.094			
Constant		-3.13 (1.13)	0	.007
TOPF-FSIQ		0.03 (0.01)	0.31	.007
Step 2	0.17			<.001
Constant		-0.2 (1.25)	0	.872
TOPF-FSIQ		0 (0.01)	-0.02	.898
WASI-II: Vocab		0.6 (0.15)	0.52	<.001
Step 3	0.178			<.001
Constant		-0.35 (1.14)	0.1	.757
TOPF-FSIQ		0 (0.01)	0.02	.851
WASI-II: Vocab		0.39 (0.14)	0.34	.009
Group: Premanifest		0.11 (0.23)	0.1	.619
Group: Manifest		-1.57 (0.39)	-1.41	<.001

Table 37: Hierarchical linear regression: Prosody-Face Matching vs WASI-II Vocabulary.

Prosody-Face Matching	(Δ)R²	B (SE)	β	p
Step 1	0.099			
Constant		-3.04 (1.11)	0	.008
TOPF-FSIQ		0.03 (0.01)	0.31	.006
Step 2	0.061			.026
Constant		-1.27 (1.33)	0	.344
TOPF-FSIQ		0.01 (0.01)	0.12	.412
WASI-II: Vocab		0.35 (0.16)	0.32	.026
Step 3	0.219			<.001
Constant		-1.28 (1.21)	0.2	.29
TOPF-FSIQ		0.01 (0.01)	0.15	.22
WASI-II: Vocab		0.09 (0.15)	0.08	.55
Group: Premanifest		-0.04 (0.24)	-0.04	.87
Group: Manifest		-1.81 (0.41)	-1.66	<.001

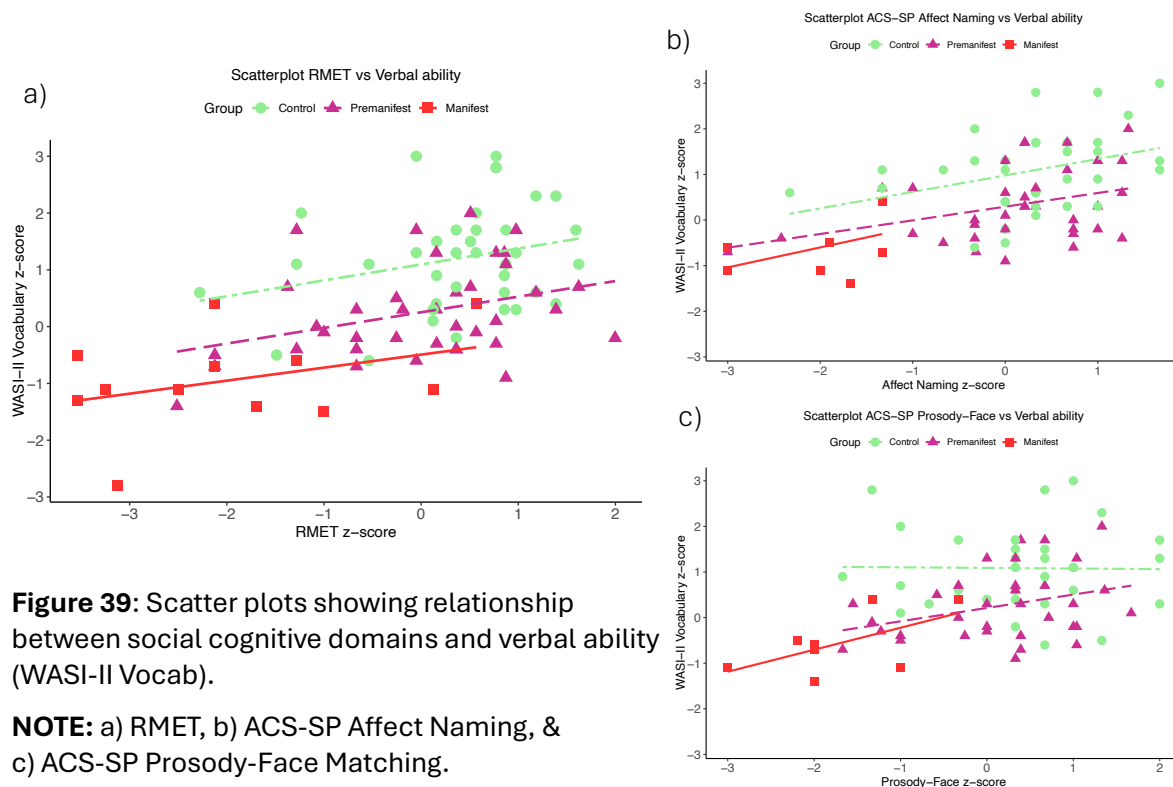


Figure 39: Scatter plots showing relationship between social cognitive domains and verbal ability (WASI-II Vocab).

NOTE: a) RMET, b) ACS-SP Affect Naming, & c) ACS-SP Prosody-Face Matching.

7.3.6 Executive function in HD

To address **Hypothesis 6.3**, this section of the study will examine performance on tasks of executive function commonly impaired in HD.

7.3.6.1 Working memory

The digit span backwards (DSB) condition showed significant differences with very large effect sizes between groups on an ANCOVA, $\eta_p^2=0.32$, $p_{\text{adj}}<.001$ (**Figure 40b**). DSB performance was lower in the premanifest group with a medium-large effect size ($d=0.62$, $95\%CI:[0.13,1.10]$), but did not reach significance after adjustment for multiple comparisons (estimate=0.57(0.22), $p_{\text{adj}}=.094$).

7.3.6.2 Simple attention & speed of processing

One-way ANCOVA found that the digit span forwards (DSF), a test of simple attention, was significantly different across the groups, $F(2,67)=3.74$, $p_{\text{adj}}=.035$, but which had a relatively small effect size compared to other measures ($\eta_p^2=0.1$), explaining only 10% of the variance in scores (**Figure 40a**). Although there was a small-to-medium effect size, planned contrasts did not indicate a significant difference between controls

and premanifest PwHD scores (estimate=0.39(0.28), $d=0.39$, $p_{\text{adj}}=.346$). Missing baseline DSF scores for several premanifest PwHD may have reduced statistical power.

Group differences on the SDMT were highly significant on the ANCOVA ($F(2,77)=31.14$, $\eta_p^2=0.45$, $p_{\text{adj}}<.001$), explaining 45% of the variance in scores. Speed of processing scores were reduced for premanifest PwHD compared to controls, but did not reach significance following adjustment (EMMs estimate=0.8(0.35), $p=.025$, $p_{\text{adj}}=.135$). This showed a medium effect ($d=0.58$, 95%CI:[0.07,1.09]). Although the manifest group made a higher number of errors on the SDMT (raw score=1.3), this did not reach statistical significance on a Kruskal-Wallis test ($H(2)=5.54(2)$, $p_{\text{adj}}=.069$).

Additional proxy measure for speed of processing include the Colour Naming condition on the CWIT (**Figure 41a**). ANCOVA, controlling for premorbid IQ, found a significant main effect of group ($F(2,85)=37.2$, $\eta_p^2=.47$, $p<.001$). Assumptions of homogeneity of regression slopes and multicollinearity were met, however, residuals showed minor deviation from normality (Shapiro-Wilk: $W=1$, $p=.005$). Nonetheless, visual inspection of residual plots indicated an acceptable model fit. Planned comparisons showed premanifest group also performed significantly worse (slower) than controls (estimate=0.9(0.26), $p_{\text{adj}}=.029$).

The same pattern for test assumption violations presented for the CWIT Reading condition (**Figure 41b**). This easier more automatic speeded task also found significant differences on the ANCOVA ($F(2,85)=47.8$, $p_{\text{adj}}<.001$), with a very large effect size ($\eta_p^2=0.53$, 95% CI[0.41, 1.00]). Controls were faster than the premanifest group, with large effect (estimate=0.87 (0.23), $d=0.89$, $p_{\text{adj}}=.017$). TOPF-FSIQ was not a significant predictor on any of these three tasks ($p>.05$).

7.3.6.3 Cognitive inhibition & flexibility (switching)

Conditions 3 and 4 of the CWIT are Inhibition and Inhibition/Switching respectively (**Figure 41c,d**). $n=3$ manifest PwHD did not complete the Inhibition/Switching condition, due to unsuccessful completion of the practice trial. Group differences in TOPF were highly predictive of scores on both conditions ($p<.05$). Both show differences across the groups on ANCOVA controlling for TOPF-FSIQ ($p<.001$) with very large ($\eta_p^2=0.51$) and large effect sizes respectively ($\eta_p^2=0.33$; **Table 38**).

These effects extended to the premanifest group, who scored significantly lower than controls on the Inhibition ($d=0.72$, 95%CI:[0.26,1.25], $p_{\text{adj}}=.049$), but not on the Inhibition/Switching condition, despite the medium effect size ($d=0.41$, 95%CI:[-0.09,0.91], $p_{\text{adj}}=.289$).

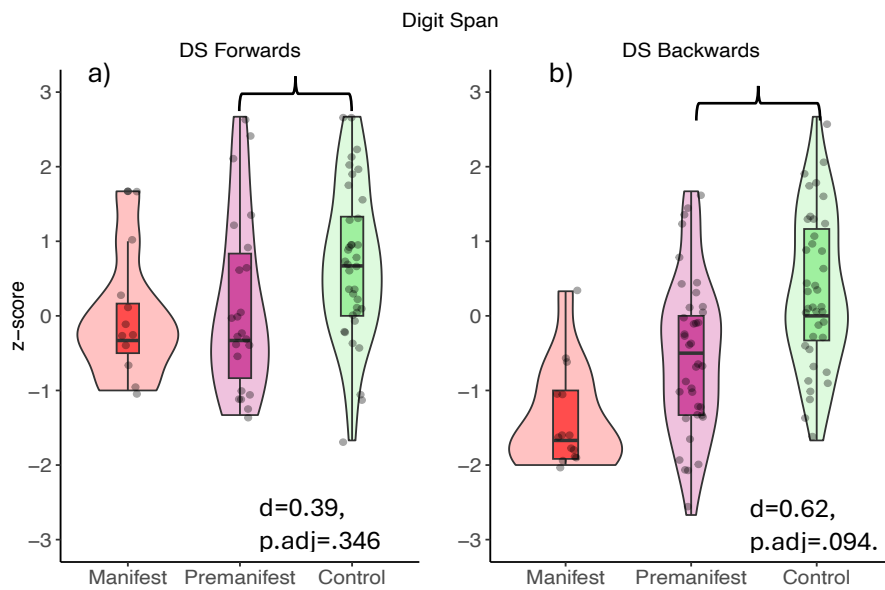
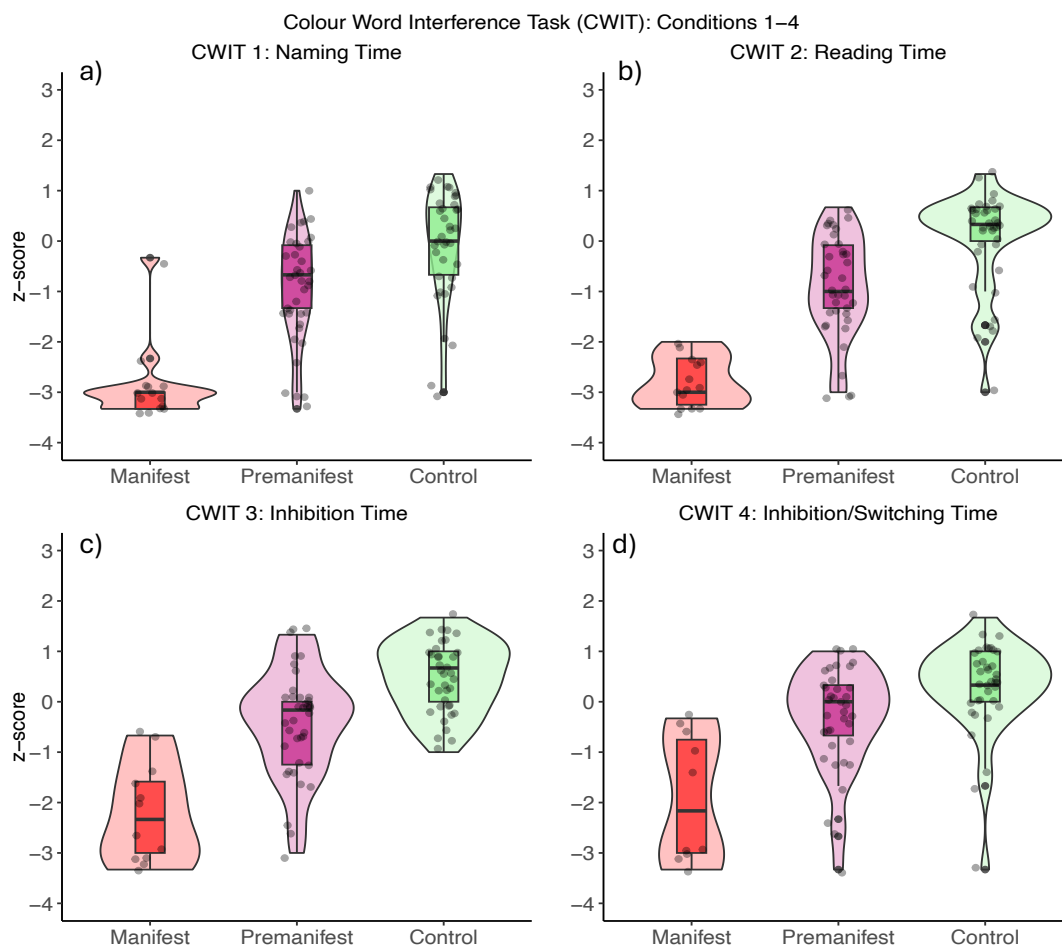


Figure 40: Violin plot for digit span forwards and backwards at baseline.

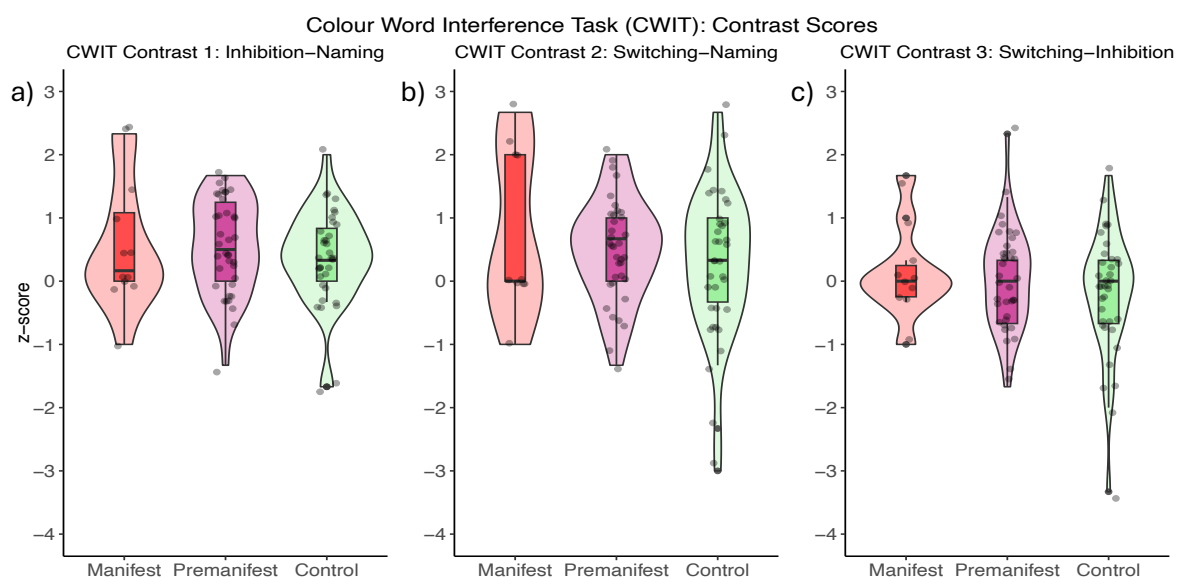
Figure 41: Executive function: The four conditions of the CWIT.



7.3.6.4 Contrast scores for executive functioning

Similar patterns of group performances are seen on all Conditions of the CWIT (Controls: $z \approx 0$; Premanifest: z between -0.33 and -1 ; manifest: z -1.9 and -2.88 ; see **Figure 41**). By considering the effects of processing speed, the underlying capacities for the more executively demanding subtasks (Condition 3 and 4), can be isolated. Standardised contrast scores for Inhibition–Naming, show no significant differences on ANCOVA ($\eta_p^2=0.004$, $p_{\text{adj}}=.862$), nor did the Inhibition/Switching–Naming ($\eta_p^2=0.02$, $p_{\text{adj}}=.51$) or Inhibition/Switching–Inhibition contrast scores ($\eta_p^2=0.02$, $p_{\text{adj}}=.48$; see **Figure 42**). The former isolates Inhibition capacity from naming speed, and the latter, the effects of mental flexibility from the previous Inhibition Condition. This indicates that cognitive processing speed accounts for the reduced scores on these tasks in PwHD rather than executive abilities (Inhibition/Switching).

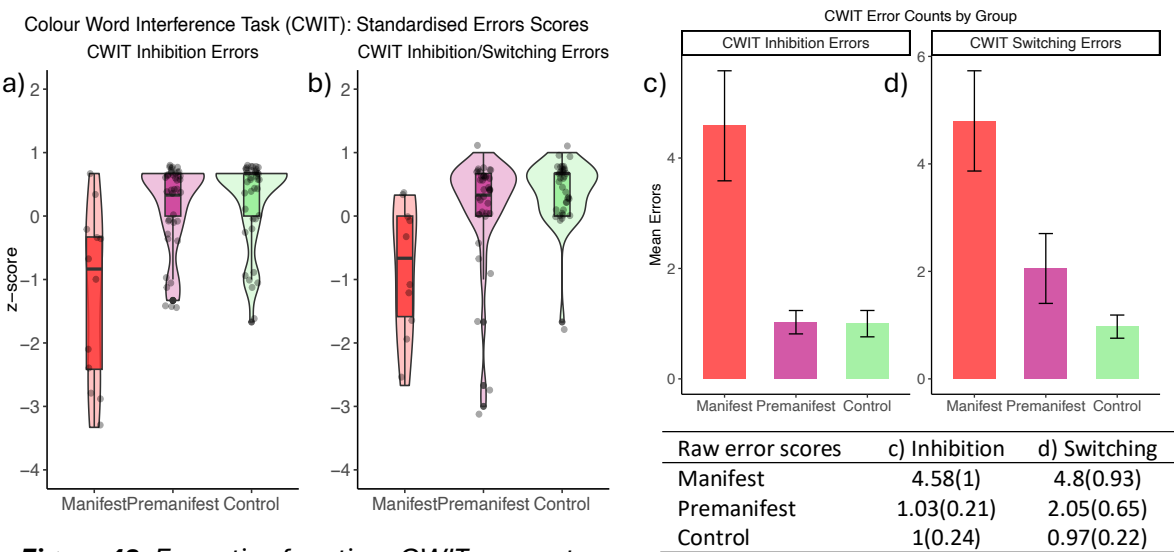
Figure 42: Executive function: CWIT domain contrast scores, showing no between-group differences.



7.3.6.5 CWIT Error patterns

Raw number of errors made on the CWIT Inhibition (Condition 3) and Inhibition/Switching (Condition 4) subtests are shown in **Figure 43c** and **d**, and the z-score equivalent in **a** and **b**. A Kruskal-Wallis test to analyse the error patterns between the groups showed PwHD made significantly more errors on the Inhibition condition ($Md_{\text{manifest}}=3.5$; $H(2)=15.53$, $p<.001$), but it was the manifest group which drove this, as premanifest and control participants did not differ ($Md_{\text{premanifest}}=1$; $Md_{\text{control}}=0$; $p=.57$). The

same pattern was observed for the Inhibition/Switching condition ($Md_{manifest}=4.5$; $H(2)=14.91, p<.001$; $Md_{premanifest}=1$; $Md_{control}=1$; $p=.3$).



7.3.7 Verbal fluency

Three verbal fluency tasks were employed in the HD-Cognition study: phonemic (FAS), semantic (animals) and restricted phonemic fluency (4-letter C; see **Figure 44**). ANCOVAs were again employed to examine overall group differences, whilst controlling for the effects of premorbid IQ estimates (TOPF-FSIQ).

Looking first at the age and education demographically-matched z-scores for FAS, a significant and very large main effect of group is seen ($\eta_p^2=0.35, p_{adj}<.001$;). There are no significant differences on planned contrasts, indicating that this effect is driven by manHD group (control vs premanifest: EMM estimate(SE)=0.3(0.22), $p_{adj}=.346$). Next, group differences in semantic fluency performance are assessed. This again showed a large significant effect ($\eta_p^2=0.33, p_{adj}<.001$) which distinguished control and preHD subjects ($p=.043$), but not after controlling for multiple comparisons (estimate(SE)=0.55(0.27), $p_{adj}=.148$).

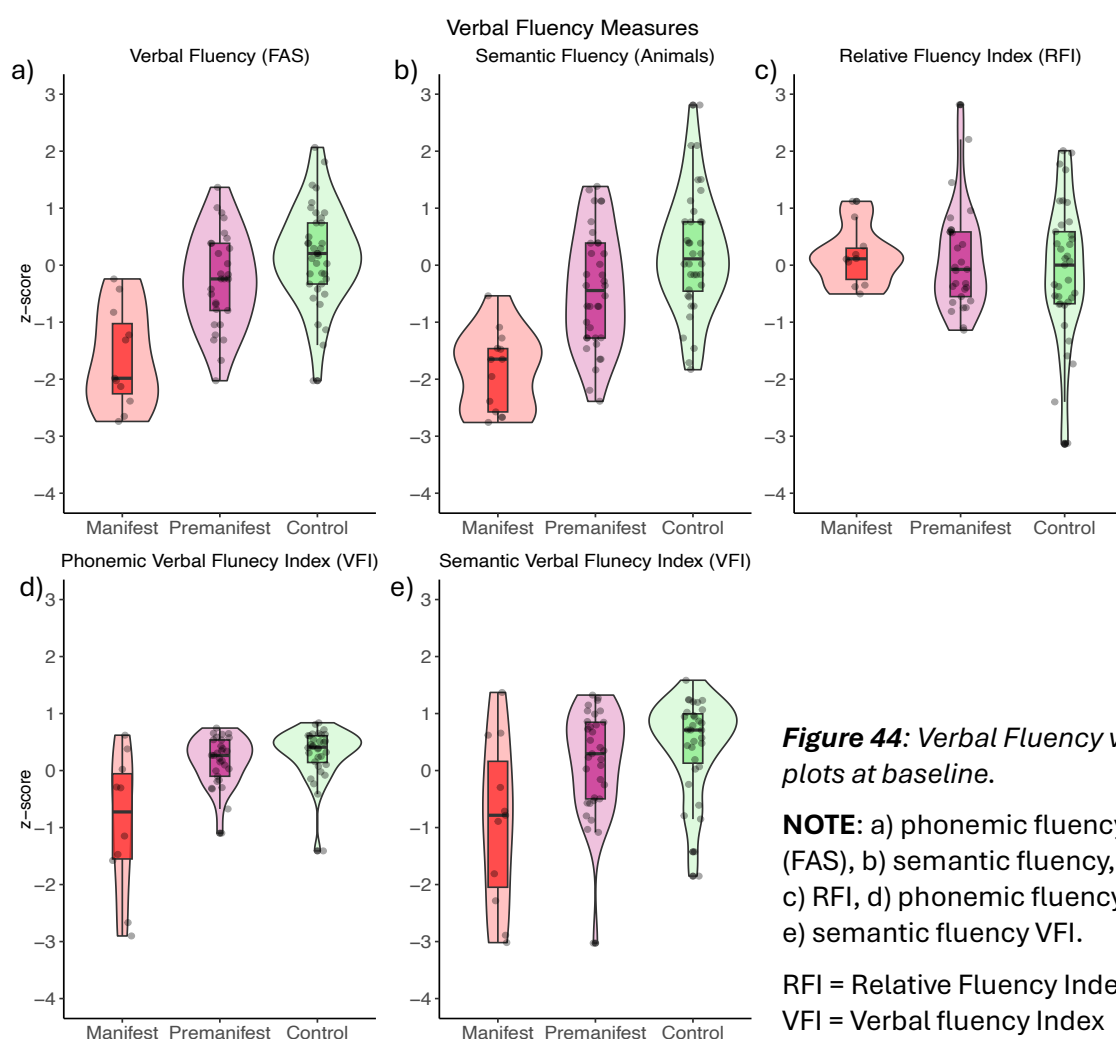
There were reduced numbers with data available for the restricted fluency paradigm ($n_{manifest}=3, n_{premanifest}=15, n_{control}=26$) and so manHD participants were not included in the analysis. There are no published normative data for the restricted paradigm, so the raw scores total correct words are compared using Welch’s t-test. It found no significant differences between the premanifest ($M(SD)=7.13(3.7)$) and control ($M(SD)=9.04(4.3)$) groups ($t(33)=-1, p=.1$).

7.3.7.1 The Relative Fluency Index (RFI)

To address **Hypothesis 7.2** as evidence for divergent validity and the utility evidenced in the previous chapter for unaffected fALS relatives, the performance of the RFI is shown here. ANCOVA revealed no significant group differences, indicative of a global, non-differentially affected performance on verbal fluency tasks, supporting the hypothesis ($\eta_p^2=0.02$, $p_{\text{adj}}=.559$; **Figure 44c**).

7.3.7.2 Controlling for verbal motor impairment: the verbal fluency index

To assess if control for motor function improves performance for the manifest and premanifest groups, the verbal fluency index (VFI) is calculated for each verbal fluency task. Linear mixed effects models (LMMs) with random intercept for participant were used to examine if group differences in verbal fluency performance differed between the methods (uncorrected age- and education-stratified z-scores vs VFI). This method allows comparison of between-subject effects and within-subject variation across the two verbal fluency outcomes.



7.3.7.2.1 *Phonemic VFI*

For phonemic fluency (FAS), the model revealed significant main effects of group, with both the premanifest ($B=1.35$, $p<.001$) and control groups ($B=1.81$, $p<.001$) scoring significantly higher than the manifest group, as above. Although VFI scores tended to be higher (less impairment; $B(SE)=0.292(0.199)$), there was no significant main effect of score type (standard vs VFI: $p=.15$), suggesting that the pattern of group differences was consistent across both scoring methods.

However, within-group contrasts showed that the premanifest group exhibited a significant difference between scoring methods, VFI significantly increasing FAS scores (estimate= 0.44 , $p=.001$). No significant differences were observed within the manifest (estimate= 0.29 , $p=.15$) or control groups (estimate= 0.11 , $p=.33$). This suggests the VFI scoring method may slightly elevate fluency scores in the premanifest group specifically, though overall group comparisons do not change with scoring method.

7.3.7.2.2 *Semantic VFI*

For semantic fluency, the model replicated the significant main effects of group shown above in univariate analyses. VFI scores tended to be higher overall ($B(SE)=0.37(0.22)$), but there was no significant main effect of score type ($p=.11$). Within-group contrasts showed that the premanifest group had significantly improved scores using the VFI method (estimate(SE)= $0.57(0.14)$, $p<.001$). No significant differences were observed within the manifest (estimate(SE)= $0.37(0.22)$, $p=.108$) or control groups (estimate(SE)= $0.12(0.14)$, $p=.395$). The VFI method may again enhance semantic fluency scores in the premanifest group specifically, but does not meaningfully alter group comparisons overall.

7.3.7.2.3 *Restricted VFI*

The VFI was calculated from the restricted paradigm, but was not available for all manifest participants at baseline. Nor are there published normative data for this score, and so z-scores cannot be computed. Comparing the two groups, VFI scores violated the assumption of normality. Even though the assumption of homogeneity of variances was met, a t-test may not be appropriate in this smaller sample. Therefore a Mann-Whitney U test was used, which showed no difference between control and premanifest groups on restricted verbal fluency VFI ($W=234$, $p=0.3$).

Table 38: Baseline neuropsychological test scores for HD-Cognition study groups.

		Controls	Premanifest	Manifest	ANCOVA (3 groups)		Planned contrasts (Control vs Premanifest EMMs) ^g			
n		40	38	14	F/H (df)	p.adj ^b	Estimate (SE)	t (df)	p ^d	p.adj
General IQ										
TOPF-UK (Standard Score)		110.93 (10.5)	102.85 (11.44)	95.99 (10.9)	11.3(2,89) ^a	<.001 ^a	8.08 (2.48)	3.26 (89)	.002 ^a	.002* ^a
WASI-II	FSIQ-2 (Standard Score)	112.5 (10.5)	101.87 (15.13)	80.33 (12.96)	42.37(2,84)	<.001	4.69 (2.61)	1.8 (84)	.076	.23
	Vocabulary (z-score)	1.14 (0.9)	0.25 (0.82)	-0.94 (0.86)	38.61(2,85)	<.001	0.53 (0.18)	2.89 (85)	.005	.059
	Matrix Reasoning (z- score)	0.23 (0.7)	-0.05 (1.15)	-1.34 (0.99)	13.98(2,84)	<.001	-0.02 (0.22)	-0.107 (84)	.915	.915
Social Cognition										
	RMET	0.39 (0.9)	-0.02 (1.05)	-2.06 (1.36)	30.05(2,85)	<.001	0.19 (0.24)	0.76 (85)	.449	.643
ACS-SP	Affect Naming	0.29 (0.9)	0.1 (0.97)	-2.16 (0.76)	30.43(2,74)	<.001	0.05 (0.23)	0.235 (74)	.815	.85
	Prosody-Face	0.39 (0.9)	0.1 (0.87)	-1.83 (0.93)	24.02(2,71)	<.001	0.09 (0.23)	0.386 (71)	.701	.764
	Prosody-Pair total	0.87 (0.9)	0.57 (0.73)	-1.62 (1.03)	22.72(2,52)	<.001	0.25 (0.27)	0.897 (52)	.374	.592
	Social Perception	0.31 (0.8)	-0.07 (0.98)	-2.13 (0.71)	29.96(2,57)	<.001	0.56 (0.7)	0.804 (57)	.425	.62
Executive function										
SDMT	Total correct	-1.23 (1.2)	-2.01 (1.51)	-5.1 (1.54)	31.14(2,77)	<.001	0.8 (0.35)	2.29 (77)	.025	.135
	Errors (raw score)	0.55 (0.8)	0.5 (0.88)	1.3 (1.25)	5.54(2) ^c	.069	-	-	-	-
Colour Word Interference Test	Naming Time	-0.07 (1.1)	-0.97 (1.05)	-2.88 (0.78)	37.2(2,85)	<.001	0.9(0.26)	3.48 (85)	.001	.029*
	Reading Time	0.05 (1)	-0.9 (1)	-2.78 (0.5)	47.8(2,85)	<.001	0.87 (0.23)	3.85 (85)	<.001	.017*
	Inhibition Time	0.48 (0.7)	-0.41 (1.06)	-2.22 (0.98)	43.9(2,83)	<.001	0.66 (0.22)	3.02 (83)	.003	.049*
	Inhibition Errors	0.22 (0.7)	0.16 (0.67)	-1.25 (1.36)	15.53(2) ^c	.001	NP	NP	NP	.58
	Switching Time	0.29 (0.9)	-0.33 (1.02)	-1.9 (1.27)	19.79(2,80)	<.001	0.4 (0.25)	1.63 (80)	.107	.289
	Switching Errors	0.4 (0.5)	0.13 (0.89)	-0.83 (1.06)	14.91(2) ^c	.001	NP	NP	NP	.3
	Inhibition – Naming	0.45 (0.9)	0.54 (0.74)	0.55 (1.01)	0.15(2,82)	.862	-	-	-	-
	Switching– Naming	0.37 (1.2)	0.48 (0.81)	0.8 (1.3)	0.68(2,79)	.507	-	-	-	-

		Controls	Premanifest	Manifest	ANCOVA (3 groups)		Planned contrasts (Control vs Premanifest EMMs) ^g			
Digit Span	Switching – Inhibition	-0.18 (1)	0.02 (0.79)	0.13 (0.74)	0.74 (2,80)	.48	-	-	-	-
	Forward	0.71 (1)	0.13 (1.17)	-0.06 (0.81)	3.74(2,67)	.035	0.39 (0.28)	1.41 (67)	.164	.346
	Reverse	0.34 (1)	-0.54 (1.04)	-1.38 (0.69)	20.39(2,87)	<.001	0.57 (0.22)	2.54 (87)	.013	.094
Verbal Fluency										
	FAS Total	0.18 (0.9)	-0.28 (0.84)	-1.63 (0.88)	20.07(2,73)	<.001	0.3 (0.22)	1.4 (73)	.166	.346
	FAS Total (VFI)	0.31 (0.4)	0.16 (0.43)	-1.34 (1.78)	18.99(2,68)	<.001	0.09 (0.21)	0.44 (68)	.662	.736
	Category (animals)	0.32 (1.3)	-0.43 (1.03)	-1.86 (0.71)	19.89(2,82)	<.001	0.55 (0.27)	2.06 (82)	.043	.148
	Category (animals; VFI)	0.46 (0.8)	0.15 (0.92)	-1.49 (1.95)	14.19(2,73)	<.001	0.28 (0.29)	0.948 (73)	.346	.575
	RFI	-0.14 (1.3)	0.12 (0.93)	0.15 (0.52)	0.59(2,72)	.559	-	-	-	-
	Restricted fluency (C; VFI) ^f	0.31 (0.5)	-0.19 (1.4)	-1.58 (2.49) ^e	-	-	-	W=234	.3	-
	Recognition	0.03 (0.9)	-0.57 (1.5)	-2.69 (2.25)	15.67(2,82)	<.001	0.48 (0.35)	1.36 (82)	.177	.358
Memory										
CVLT	T1-5 Total	0.21 (0.5)	-0.27 (1.08)	-1.61 (0.65)	27.82(2,85)	<.001	0.49 (0.22)	2.2 (85)	.031	.136
	Immediate Free	0.31 (0.8)	-0.14 (1.15)	-1.34 (0.82)	12.85(2,60)	<.001	0.36 (0.27)	1.35 (60)	.182	.358
	Immediate Cued	0.23 (0.8)	-0.3 (1.33)	-1.49 (1.02)	11.7(2,60)	<.001	0.44 (0.29)	1.52 (60)	.135	.309
	Delayed Free	0.2 (0.7)	-0.27 (1.1)	-1.46 (1.01)	14.38(2,60)	<.001	0.4 (0.25)	1.58 (60)	.12	.293
	Delayed Cued	0.11 (0.7)	-0.52 (1.25)	-1.67 (0.79)	15.68(2,60)	<.001	0.56 (0.26)	2.13 (60)	.038	.137
	Recognition hits	-0.21 (1)	-0.44 (1.25)	-1 (0.77)	3.64(2) ^c	.185	-	-	-	-
	Recognition (discrim NP)	-0.07 (1)	-0.41 (1.01)	-1.3 (0.48)	7.06(2,59)	.007	0.23 (0.26)	0.874 (59)	.386	.592
	Intrusions free (raw score)	1.78 (1.7)	1.76 (1.51)	4.18 (3.4)	6.89(2) ^c	.051	-	-	-	-
	Intrusions cued (raw score)	1.97 (1.8)	3.1 (3.81)	7.27 (6.56)	10.39(2) ^c	.011	NP	NP	NP	.823
	Intrusions total (raw score)	5.5 (4.7)	7.14 (6)	14.91 (10.33)	10.8(2) ^c	.011	NP	NP	NP	.469
	Intrusions total	-0.23 (0.9)	-0.51 (0.9)	-1.43 (0.93)	7.42(2,60)	.007	0.14 (0.25)	0.541 (60)	.59	.727

		Controls	Premanifest	Manifest	ANCOVA (3 groups)		Planned contrasts (Control vs Premanifest EMMs) ^g			
Logical Memory	Across-within intrusions (raw score)	2.88 (3.7)	3.43 (4.15)	5.55 (4.82)	4.55(2) ^c	.137	-	-	-	-
	Repetition errors (raw score)	3.88 (3.5)	4.67 (3.76)	4.82 (3.68)	1.68(2) ^c	.431	-	-	-	-
	Immediate	0.69 (0.8)	-0.04 (1.04)	-1.64 (1.13)	27.84(2,83)	<.001	0.51 (0.24)	2.17 (83)	.033	.136
	Delay	0.57 (0.9)	-0.25 (0.94)	-1.72 (1.03)	30.15(2,83)	<.001	0.58 (0.22)	2.56 (83)	.012	.094
	LM retention (%)	84.61 (10.3)	78.66 (14.82)	62.38 (26.24)	12.47(2) ^c	.002	NP	NP	NP	.037
RCFT	Recognition (raw)	25.46 (2.5)	24.3 (3.33)	18.75 (4.09)	21.68(2,82)	<.001	0.72 (0.77)	0.935 (82)	.353	.575
	Copy score	-0.45 (1.3)	-0.89 (2.21)	-5.06 (3.42)	22.26(2,82)	<.001	0.06 (0.51)	0.113 (82)	.91	.915
	Immediate recall	0.3 (1.3)	-0.1 (1.51)	-1.7 (1.05)	10.04(2,80)	<.001	0.08 (0.33)	0.242 (80)	.81	.85
	Delayed recall	0.22 (1.3)	-0.22 (1.56)	-1.55 (0.85)	7.46(2,80)	.001	0.15 (0.33)	0.434 (80)	.666	.736
	Recognition	0.03 (0.9)	-0.57 (1.5)	-2.69 (2.25)	15.67(2,82)	<.001	0.48 (0.35)	1.36 (82)	.177	.358
Language										
Sydney	Naming	0.51 (0.8)	-0.29 (1.17)	-2.75 (1.49)	42.03(2,81)	<.001	0.67 (0.25)	2.67 (81)	.009	.094
Language	Comprehension	0.28 (0.8)	0.08 (1.27)	-2.14 (1.26)	19.74(2,57)	<.001	0.05 (0.28)	0.174 (57)	.863	.887
Battery	Semantic	0.35 (1.2)	0.06 (1.66)	-2.44 (1.53)	13.94(2) ^c	.001	NP	NP	NP	.633
Cognitive Reserve (CRIq)										
(converted score)										
	Education	111.24 (7.4)	110.45 (8.77)	106.2 (4.26)	1.68(2,70) ^a	0.19 ^a	-	-	-	-
	Working	103.94 (8.8)	104.14 (10.77)	104.4 (14.9)	0.001(2,69) ^a	0.99 ^a	-	-	-	-
	Leisure	111.41 (14.3)	113.38 (13.82)	107.6 (15.92)	0.61(2,70) ^a	0.54 ^a	-	-	-	-
	Total	111.67 (10.3)	112.38 (10.54)	107.9 (12.02)	0.67(2,69) ^a	0.51 ^a	-	-	-	-

NOTE: Test scores are converted to z-scores and given as Mean(SD), unless otherwise stated. All tests are computed with TOPF as a covariate, with the exception of TOPF itself, and CRIq variables.^a ANOVA with no covariates; ^b Benjamini-Hochberg correction for multiple comparisons applied within cognitive domain; ^c Non-Parametric Kruskal-Wallis test; ^d unadjusted p-value for planned comparisons (Controls – Premanifest); ^e manifest group excluded from analysis due to low numbers of available data (n=3); ^f locally generated control sample data; ^g EMM = Estimated marginal means used in planned contrasts. They show the effect of group, controlling for TOPF-FSIQ. Significant p-values in bold. * significant p-values for controls vs premanifest after correction for multiple comparison. NP=non-parametric; therefore some statistics not computable.

7.3.8 Other cognitive domains

Results for the assessment of memory and language tasks are also shown in **Table 38**. Results will not be commented on for the purpose of this chapter.

7.3.9 Questionnaire data

Outcomes of questionnaire data are presented, with ANCOVA examining group differences whilst controlling for age. Numbers are reduced for self-report questionnaire data, and much reduced for companion-rated scales. Results are shown in **Table 39**.

7.3.9.1 Mood measures

The Hospital Anxiety and Depression Scale (HADS-R), which minimises the effects of motor dysfunction, was administered to each participant. Groups differed significantly on the Anxiety ($\eta_p^2=0.11$, $p_{\text{adj}}=.036$) and Depression subscales ($\eta_p^2=0.18$, $p_{\text{adj}}=.006$). Both had large effect sizes. There was a trend for premanifest PwHD to score higher than controls, but did not reach significance on planned contrasts ($p_{\text{adj}}=.083$).

7.3.9.2 Disability

The WHO-Disability Assessment Schedule (WHODAS) has 7 domains which combine to give a total score. All variables on this scale violated assumptions of normality and variance of the residuals, and so Kruskal-Wallis H-tests were used to evaluate group differences.

On the overall WHODAS-Total score, groups were significantly different, with a large effect size ($\epsilon^2=0.173$, $p_{\text{adj}}=.006$).

Table 39 shows that premanifest participants rated themselves as significantly less disabled than manifest participants, and trended towards higher scores than controls ($p_{\text{adj}}=.135$), on a post-hoc pairwise independent Mann-Whitney U/Wilcoxon rank-sum test. The large SD in the premanifest group evidences high variation in ratings. The largest ϵ^2 values for the WHODAS were on the Participation in Society ($\epsilon^2=0.24$, $p_{\text{adj}}=.003$), Self-Care ($\epsilon^2=0.233$, $p_{\text{adj}}=.003$), Getting Around ($\epsilon^2=0.199$, $p_{\text{adj}}=.005$) and Understanding and Communicating ($\epsilon^2=0.16$, $p_{\text{adj}}=.01$). Only Participation in Society was significant in post-hoc comparisons, with premanifest group scoring significantly higher than the control group ($p_{\text{adj}}=.006$).

7.3.9.3 Fatigue

In the baseline sample overall, PwHD experienced higher levels of fatigue across all of the domains of the Multidimensional Fatigue Symptom Inventory–Short Form (MFSI-SF). ANCOVA, controlling for the effects of age, found no differences between groups on the Vigour subdomain ($\eta_p^2=0.019$, $p_{\text{adj}}=.587$). However, groups differed on each of the other sub-domains (General, Physical, Emotional, Mental; $p<.05$), with large effect sizes ($\eta_p^2=0.11\text{--}0.35$). The largest effect size was for the Mental fatigue subdomain ($\eta_p^2=0.346$, $p_{\text{adj}}<.001$).

The premanifest group endorsed significantly more symptoms of fatigue than control participants in planned contrasts. However, after correcting for multiple comparisons, General and Physical fatigue were no longer significant ($p_{\text{adj}}>.05$). Remaining significant were, Emotional ($d=0.90$, 95%CI:[0.29,1.52], $p_{\text{adj}}=.015$), Mental ($d=0.97$, 95%CI:[0.35,1.59], $p_{\text{adj}}=.015$), and Total Fatigue ($d=0.84$, 95%CI:[0.25,1.44], $p_{\text{adj}}=.017$) with large effect sizes.

7.3.9.4 Quality of life

Health-related QoL was assessed using the EQ-5D-3L (EuroQoL). The sixth question, assessing a respondent's current perceived health state, violated assumptions of the ANCOVA and so a Kruskal-Wallis H-test was employed. Although the manifest group endorsed lower levels of health-related QoL (59.55% of optimal) for a small-to-medium effect size, there were no significant group differences (Control=78.74%; Premanifest=77.52%; $H(2)=4.66$, $p_{\text{adj}}=.097$). Manifest and premanifest group standard deviations were high, indicating high within group variability.

7.3.9.5 Behaviour

The BBI, an informant-rated behavioural questionnaire, was used to quantify behavioural change in participants. Power is reduced for this analysis as a large number, particularly premanifest participants, did not have BBI data available (see **Table 39**). These data are missing mostly at random, and so still warrant comparison. Companions rate participants' behaviour before and after motor-symptom onset, or in the case of premanifest and control groups, 10 years ago and in the last 3 years.

Assumptions of the ANCOVA were violated on both pre and post scales, and so Kruskal-Wallis H-tests were performed. There were significant differences with large and very large effect sizes on the 'pre' ($\epsilon^2=0.33$, $p_{\text{adj}}=.004$) and 'post' scales ($\epsilon^2=0.59$, $p_{\text{adj}}<.001$). The difference between 'pre' and 'post' scores (post–pre), an indicator of

recent change from baseline, also showed a very large effect size on the Kruskal-Wallis H-test ($\varepsilon^2=0.61$, $p_{\text{adj}}=.001$). Post-hoc Wilcoxon rank-sum tests after correction for multiple comparisons found differences for controls and premanifest PwHD on all BBI scores, but only the difference score (post–pre), remained significant after correction ($p_{\text{adj}}=.036$). This indicates that the premanifest group experienced significantly more changes to their behaviour in recent years than the controls ($p_{\text{adj}}=0.36$).

Group scores were also compared on rates of impairment on the BBI using validated clinical cut-offs (**Figure 45b**).⁵⁷⁸ All manifest participants (100%) are rated by a companion as surpassing the cut-off for severe behaviour change post-motor symptom onset, compared to ($n=2$) 33.3% of premanifest and 0% of controls in the preceding 3 years. A total of 66.7% of premanifest participants were rated as experiencing mild behaviour change, compared to 18.8% of controls in the past three years.

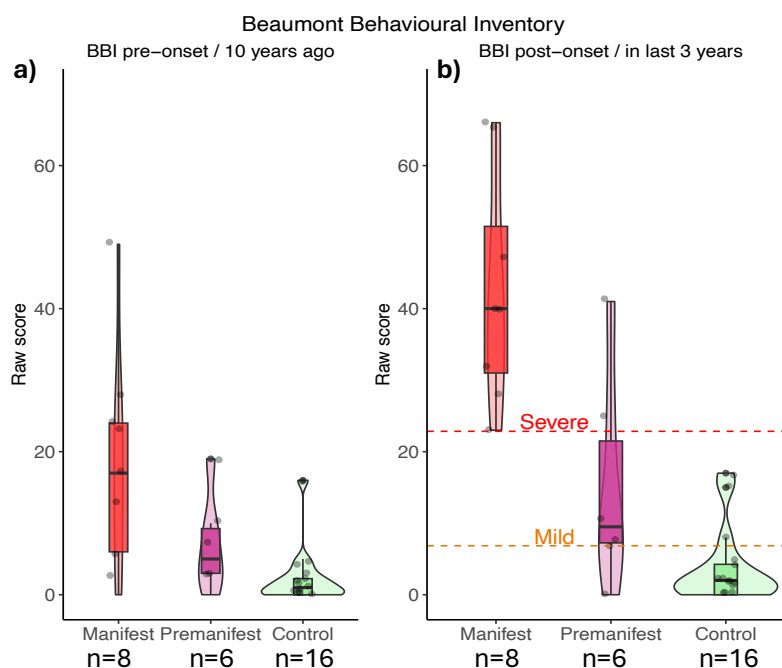


Figure 45: BBI behavioural scores pre-/post-onset.

Table 39: Questionnaire data: Measures of mood, disability and behaviour at baseline in HD-Cognition study

		Controls	Premanifest	Manifest	ANCOVA (3 groups)			Planned contrasts (Control vs Premanifest EMMs) ^c			
Questionnaires (n)		38	28	12	F/H (df)	p	p.adj	Estimate (SE)	t	p	p.adj
Mood	HADS-anxiety	4.86 (2.8)	6.65 (2.81)	8.4 (5.99)	3.74 (2,58)	.03	.036^a	1.91(1.01)	1.89(58)	.064.	.083
	HADS-depression	2.1 (2.1)	3.65 (2.6)	5.5 (2.95)	6.53 (2,58)	.003	.006^a	1.37(0.724)	1.89(58)	.064.	.083
Disability	WHODAS Total	40.43 (5.6)	47.32 (15.58)	74.6 (27.69)	11(2)	.004	.006^a	-	-	.108	.135
	Understanding and Communication	7.07 (1.1)	8.61 (2.99)	13.2 (5.45)	10.3(2)	.006	.01^b	-	-	.171	.205
	Getting Around	5.43 (0.6)	6.26 (3.25)	11.3 (5.31)	12.4(2)	.002	.005^b	-	-	.584	.626
	Self-care	4.14 (0.4)	4.1 (1.45)	7.1 (3.28)	14.1(2)	.001	.003^b	-	-	.874	.889
	Getting along with People	6.14 (1.7)	6.61 (2.69)	9.1 (4.7)	4.37(2)	.113	.132 ^b	-	-	NS	NS
	Household Activities	5.07 (1.7)	5.29 (2.33)	10.5 (6.38)	6.51(2)	.039	.054. ^b	-	-	.53	.584
	School/Work Activities	4.17 (0.6)	5.67 (3.23)	8 (4.62)	3.97(2)	.138	.138 ^b	-	-	NS	NS
	Participation in Society	9 (1.5)	12.07 (3.82)	20.2 (9.18)	14.5(2)	.001	.003^b	-	-	.003	.006*
Fatigue	MFSI General	5.32 (3.4)	8.37 (5.76)	10.73 (7.51)	3.6 (2,56)	.034	.042^b	3.12(1.63)	1.92(56)	.06.	.083
	MFSI Physical	1.05 (1.9)	4.23 (4.58)	9.18 (7.44)	10.5 (2,56)	<.001	<.001^b	3.21(1.38)	2.33(56)	.023	.052.
	MFSI Emotional	2.58 (2.3)	6.5 (4.13)	6.36 (7.08)	5.05 (2,56)	.01	.016^b	3.98(1.29)	3.08(56)	.003	.015*
	MFSI Mental	1.32 (1.7)	6.37 (4.77)	11.82 (8.86)	14.8 (2,56)	<.001	<.001^b	5.01(1.52)	3.3(56)	.002	.015*
	MFSI Vigour	12.9 (4.9)	11.33 (5.03)	12.18 (6.19)	0.537 (2,56)	.587	.587 ^b	-	-	NS	NS
	MFSI Total	-2.63 (10.9)	14.13 (19.04)	25.91 (30.67)	7.96 (2,56)	.001	.003^a	16.7(5.83)	2.87(56)	.006	.017*
Quality of life	EuroQoL	78.74 (11)	77.52 (14.28)	59.55 (27.05)	4.66(2)	.097	.097 ^a	-	-	NS	NS
Companion questionnaires (n)		16	6	8							
Behaviour	BBI pre / 10 years ago	2.31 (4)	7 (6.84)	18.11 (15.17)	11.2(2)	.004	.004^b	-	-	.047	.062
	BBI post / last 3 years	3.75 (5.3)	15.33 (15.03)	42.63 (16)	17.9(2)	<.001	<.001^b	-	-	.055	.071
	BBI Difference	1.44 (3)	8.33 (11.29)	22.63 (15.79)	18.4(2)	<.001	<.001^a	-	-	.024	.036*

NOTE: ^a Total / domain scores corrected for multiple comparisons; ^b Sub-domains corrected for multiple comparisons within test; ^c EMM = Estimated marginal means used in planned contrasts. They show the effect of group, controlling for age. Parametric comparisons (ANCOVA) control for the effects of age. Non-parametric use Kruskal-Wallis.

EuroQoL = EQ-5D-3L Health-related quality of life measure. *BBI* = *Beaumont Behavioural Inventory*.

MFSI Total: First four domains minus 'Vigour' domain to give total measure of Fatigue.

Significant p-values in bold. * significant p-values for controls vs premanifest after correction for multiple comparison.

7.4 Discussion

This chapter presented a baseline analysis of the HD-Cognition study, examining the neuropsychological profiles of PwHD, with a focus on social cognitive function and executive contrast scores. Aiming to determine the discriminatory power of a new battery of social cognitive tests against the established RMET, with findings suggesting they tap a shared underlying construct. Performance was associated with verbal ability and, also disease progression estimates. The confounding effects of speed of processing on timed-tasks of executive function in HD are demonstrated. Highlighting the early and broad impact of HD on psycho-social wellbeing, analysis of questionnaire data finds group-level increases in fatigue and behavioural symptoms, but not anxiety or depression in premanifest participants.

7.4.1 *Social cognitive function in HD*

The ACS-SP is a short battery assessing emotion recognition through facial affect, prosody, and their combination, as well as more nuanced social cues. Reductions on all social cognitive tasks are evident in the manHD group. Planned contrasts showed that, although the premanifest group trended slightly lower on the RMET and ACS-SP subtests, there were no significant difference to control participants. Whereas nearly 70% of manifest participants were below clinical-cut offs on the RMET, only slightly elevated rates are seen (7.9%) in premanifest PwHD. Predictive ability improved slightly, but at the cost of specificity with the use of AUC-derived optimal thresholds. These findings do not support the hypothesis that preHD participants show an intermediate profile on social cognitive tasks. This finding is in agreement with a recent study by Calliaud et al.³⁶⁵ and others which found no differences in emotion recognition scores in preHD.

Affect recognition is shown to relate to disease onset in premanifest PwHD, and progresses linearly past motor-symptom onset in association with amygdala volume.⁶⁸² This association was not evidenced by the ACS-SP affect naming task, despite its high association to the other social cognition tasks, which did. Looking at ACS-SP subtest scores in the premanifest group alone, grouped by estimates of time to predicted diagnosis (far, near, past), Affect Naming performance differentiated the groups, with those 'past' probabilistic estimates of AoO significantly lower than those estimated to be near and far from diagnosis. CAP100 only explained 6% of the variance in Affect

naming performance in the regression model, but demonstrated a very large effect in group comparisons ($\eta^2=0.30$), although not statistically significant. Together this indicates that the relationship is not linear for those prior to estimated diagnosis (far and near groups), but decreases for some participants in the peri-manifest stage as CAG-associated probabilities indicate individuals should have developed motor symptoms (see **Figure 36**).⁷¹ Importantly, these participants were all clinically premanifest, having not received clinical motor diagnosis ($DCL < 3$).

On the other hand, the strong negative linear relationship between the Prosody-Face Matching subtest and CAP100 scores, as well significant premanifest group differences with large effects ($\eta^2=0.27$) suggest that this subtask may provide more sensitivity to decline across the premanifest phase. Although not statistically significant, effect size was also large between premanifest and control participants for the more complex ACS-SP Prosody-Pair subtest ($d=0.275$), which requires multiple levels of social cognitive processing. This does not provide support for **Hypothesis 5.2**, as, although a more challenging task, it was hypothesised that the extra social cues and context might have facilitated performance for PwHD compared to the more unimodal tasks (e.g.: RMET).⁶⁹⁶ Groups were smaller for these latter subtests due to missing data however and longitudinal trajectories discussed in the next chapter may shed more light on this.

Significant differences between premanifest and control groups were not evident on the RMET, which showed a smaller effect size ($d=0.186$), but did demonstrate a linear relationship with CAP100 scores through the premanifest stage, explaining 18% of the variance. RMET also differentiated premanifest groups significantly on proximity to diagnosis. This is in contrast to Turner et al. (2022)³⁵⁴, who demonstrated very large effect sizes between controls and premanifest PwHD on the RMET, but also other tasks of ToM, social perception and empathy with 22% falling below clinical cut-offs in contrast to the 7.9% here.

7.4.1.1 Verbal ability & RMET performance

Hierarchical linear regression revealed that verbal ability influenced performance differentially across the three social cognition tasks: the RMET, ACS-SP Affect Naming, and Prosody-Face Matching. It was hypothesised that RMET performance would be adversely and significantly affected by verbal ability, above that of the other subtests. However, the standardised regression coefficients from Step 3 across models indicate that Vocabulary was most strongly associated with Affect

Naming performance ($\beta=0.34$), followed by RMET ($\beta=0.22$), and showed minimal association with Prosody-Face Matching ($\beta=0.08$), when accounting for group and premorbid IQ estimates. Concordant with its design and the hypothesis that it would have the lowest demands on verbal ability, Prosody-Face matching was least influenced by Vocabulary scores and may be particularly suitable for use in populations with low verbal ability or reading impairments.

Unexpectedly, verbal ability remained a significant independent predictor of test performance in the Affect Naming model, despite the commonality and high-frequency of words used to identify the basic emotions in this task. In contrast, although related, verbal ability was not a significant predictor of RMET performance. This suggests a modest and linear contribution of verbal ability to performance on the RMET, which includes lower-frequency emotion and mental-state descriptors such as “pensive,” “contemplative,” or “sceptical.” The RMET remained sensitive to group differences (manifest vs. control) even after adjusting for Vocabulary, which supports its utility in detecting social cognitive impairments related to neurodegenerative disease. Findings partially support previous studies citing an overreliance on linguistic ability for the RMET^{679,685}, and indicates that relationship to verbal ability may be linear, but not categorical, and therefore there is no clear evidence in support for a threshold or cut-off below which performance drops off in this sample. Findings suggest that crystallised verbal ability plays an important role in the accurate interpretation of emotional expressions, even when linguistic demands are relatively basic. The strong association to verbal ability here is in contrast with other studies, which show weak associations in disorders which affect global intellectual functioning to a lesser degree than HD.⁶⁹⁷

Global factors of crystallised intelligence captured by the WASI-II Vocabulary subtest, above that of verbal ability alone, may underscore the associations in this sample, where IQ also differs between the groups. The inclusion of TOPF-FSIQ as a covariate endeavoured to control for these effects however, and is evidenced by the shared variance in subsequent steps of the hierarchical regressions. manHD individuals had equivalent levels of education to preHD, and although they were older, the resultant standard and z-scores control for age in the ACS-SP tasks. In any case, the ACS-SP Prosody-Face Matching task is less influenced by verbal ability, making it a suitable tool for comparing groups with varying levels of language proficiency or neurodegeneration-associated declines to more global function.

7.4.1.2 Theory of Mind or emotion recognition

Although the RMET is a widely used task of ToM, it was shown to be highly related to the two of the affect recognition ACS-SP subtests examined here (from words and prosodic speech). Affect naming and RMET performance were most highly correlated ($r=0.696$) and factor analysis found only one significant shared factor between the three tests. Other studies have reported similar limitations to the RMET as a test of ToM. ToM, as a distinct cognitive construct is not explained in terms of other executive or emotion recognition tasks⁶⁹⁸, conditions which the RMET may not meet.

The RMET may not show sufficient construct validity for affective ToM above affect recognition. Alternative tests, such as the Edinburgh Social Cognition Test (ESCoT)⁶⁹⁹, and the mini-Social cognition and Emotional Assessment (mini-SEA), which combines facial affect recognition and the Faux Pas test of ToM, have minimal reliance on language. They are more direct assessments of broad social cognitive function which focus on ToM, show utility across levels of verbal ability. Although they have shown diagnostic utility and validity, they do not yet have robust psychometric properties in English.⁷⁰⁰ The Awareness of Social Inference Test (TASIT) does, but is more language dependent.

Demonstrating an association between cognitive function, and predicted disease onset and CAP100 score are key findings in the premanifest group, adding to the conflicting findings in the wider field. The research supports initiatives in HD^{307,308,310} and NCDs³⁶⁶ more generally, where definitive clinical recommendations and validated assessments of social cognitive function are needed. The current results are cross-sectional however. Longitudinal change is addressed in the next chapter.

7.4.2 Executive function in HD

Manifest and premanifest groups demonstrate executive deficits at the group level on outcomes analysed here, extending to tasks of response inhibition and mental flexibility/switching in preHD (CWIT). However, isolating these particular functions (inhibition/flexibility) by factoring out the speed component revealed no significant group differences, in manifest or premanifest PwHD. Reduced processing speed rather than core executive dysfunction underlies performance deficits on these tasks.

Whilst the DSF task of simple attention revealed small between-group effect sizes ($\eta_p^2=0.1$), a dissociation emerged with the more executively-demanding working memory task of the backwards condition. It requires additional mental manipulation

and retention in working memory, and showed significant differences with very large effect sizes between groups ($\eta_p^2=0.32$). These effects were also higher in the premanifest group than for DSF ($d=0.62$ vs 0.39 respectively), which approached significance.

Verbal fluency was significantly impaired in manHD. Hypothesising that controlling for motor-dysfunction in manHD would ameliorate these deficits to a degree, LMMs determined that VFI-adjusted scores did not change between-group patterns. The VFI improved scores in both semantic and phonemic fluency tasks in the premanifest group however. As this group did not present with motor symptoms, this may reflect a speed of processing effect, marginally reduced in this group, whereby premanifest participants had slower times to read aloud the words, not due to motor dysfunction, but reduced speed of processing.⁶⁹³ This was not apparent in manHD due to the floor effects and significant reductions in task performance shown.

Error patterns confirmed that manHD showed significantly more errors than controls on CWIT Inhibition task, but not on the SDMT. Only slightly more errors were made, with no differences at all between premanifest and control groups, in contrast to the large effects on the speeded outcome measures. Error monitoring is a core executive function, underwritten by right lateral PFC function.^{333,701} Whilst processing speed is related to atrophy of fronto-parietal cortex and striatum directly⁷⁰², executive deficits in HD (and ALS) are primarily caused by fronto-striatal network dysfunction.^{178,251,501} This might imply that primary striatal degeneration may exert a stronger influence on cognitive outcomes than more distributed network-level disruption in the dorsolateral-striatal circuit.³³² This is evidenced by the marked slowing in processing speed, accompanied by relatively subtle deficits in error-monitoring, particularly in early disease stages.

Executive dysfunction is evident in HD above that of speed of processing, detectable on set-shifting tasks (error rates) in far-from onset premanifest PwHD.⁴⁹¹ Evidenced here by decreased accuracy/increased errors for manHD and impaired working memory ability on non-timed task (DSB). Comparing this profile to Stuss' model of the executive functions³³², it would suggest impairment of super-medial pathway with poorer intrinsic response generation, reduced error-monitoring (SDMT/CWIT) suggests dorsolateral-striatal circuit impairment, but to a lesser degree and global increases in behaviour symptoms, and reduction in tasks of social cognitive performance implicates orbitofrontal circuits. In support of **Hypothesis 5.1**, premanifest participants show intermediate cognitive performance executive function tasks, but mostly related to

speed. Trends were present on social cognitive tasks, that were significantly related to CAP100-estimated disease progression, also supporting this hypothesis.

7.4.2.1.1 The RFI in HD – evidence of discriminant validity

In line with **Hypothesis 7.2**, and longitudinal evidence from Ho et al. (2002)¹⁴⁰, the RFI did not differentiate PwHD groups, evidencing a global non-differentially affected performance on phonemic and semantic fluency tasks.

7.4.2.2 Fatigue & behaviour

Informant-rated behavioural assessments indicated significant behavioural changes in both premanifest and manifest groups compared to controls, with very large effect sizes, although numbers with complete data were much reduced. Notably, premanifest participants showed significantly more behavioural changes ‘in the last three years’ than controls, with 33.3% (2/6) surpassing thresholds for severe behavioural dysfunction, indicative of support for the development of behavioural symptoms early in premanifest PwHD.³⁷⁸

Significantly higher symptoms of pervasive fatigue experienced in the past week were endorsed by premanifest participants compared to controls. The highest effect sizes were for Mental and Emotional fatigue ($d > 0.9$). Fatigue is a common symptom of many psychiatric and neurological disorders, and can be debilitating, often impacting quality of life⁷⁰³, but its effects on cognitive function are less clear.⁷⁰⁴⁻⁷⁰⁶ The association between fatigue, apathy and other behavioural and cognitive test scores is explored in the next chapter.

7.4.3 Study-specific limitations

As with many past studies, this study is afflicted by methodological limitations in studying social cognition in preHD, including a relatively small sample size.^{358,364} Aiming to improve sensitivity at the group level, premanifest PwHD were stratified by probabilistic proximity to diagnosis⁷¹ and linear associations to CAP100 score.

Omitted tests, systematic errors in administration and the introduction of new tests to the protocol contribute to missing data in the baseline HD-Cognition study, and reduce the strength of the conclusions possible here. Many of the participants did not complete the full ACS-SP battery at T1. Therefore sub-group analyses were not possible due to the small numbers in the groups for those subtests. This can be seen by reduced degrees of freedom in some of the analyses. Data missing not-at-random (MNAR) were

also present for a number of tests, as manHD participants could not complete them due to reduced function (i.e.: ACS-SP Prosody-Pair subtask, SDMT, CWIT Condition 4). This was relatively uncommon, but likely underestimates impairment at the group level on these tasks in the manifest group, as well as reducing statistical power.

7.5 Conclusions

Social cognitive function did not distinguish premanifest PwHD from controls, but linear performance reductions with CAG-associated estimates of disease progression in the premanifest group were evident. RMET performance was predicted by verbal ability when controlling for premorbid IQ estimates, but group effects were superior, although not significant in preHD. This effect extended beyond RMET alone however to facial-affect recognition, where verbal ability significantly impacted performance. Other issues with the RMET arose through factor analysis. Whilst the ACS-SP shows similar discriminatory power to the RMET demonstrating high construct validity, the RMET itself did not exhibit an additional latent factor beyond affect recognition. This has implications for its consideration as a test of affective ToM.

Large effects of speeded tasks of executive function are attenuated by controlling for general processing speed in manifest and preHD. Increased error rates remain an indicator of executive task-monitoring, as does the backwards digit span working memory task of mental flexibility, which had a trend level effect in preHD. Behavioural and fatigue symptoms were also evident in a subset of premanifest participants, supporting the thesis that the extramotor phenotype of HD begins early. These results have important implications for the development and interpretation of cognitive biomarkers and clinical extramotor diagnostic criteria in HD, underscoring the importance of accounting for demographic and processing speed confounds for latent construct validity. Longitudinal data will further explore these relationships and their clinical utility in premanifest and early-stage HD.

8 Chapter 8:

Results: A HD-Cognition longitudinal study

8.1 Introduction

This chapter continues the analysis of the HD-Cognition study data, introduced in the last chapter, to investigate the longitudinal trajectories of key cognitive domains of interest in premanifest and early-HD. Building on the cross-sectional findings presented previously, this chapter evaluates the degree to which subclinical changes in executive function and social cognition progress over time in PwHD prior to clinical diagnosis and around the peri-manifest period. Studies are needed which interrogate this phase to support the creation and standardisation of extramotor diagnostic criteria^{223,308}, and identify sensitive cognitive biomarkers of change for prognostication and evaluation of outcomes from clinical trials.^{237,256,279}

Complete penetrance of *HTT* and the association of disease factors to repeat length afford a unique opportunity to longitudinally track the evolution of symptoms through the presymptomatic and prodromal phases of disease in HD. Due to the variability in disease onset, and extramotor symptom profile in premanifest and early HD, cross-sectional studies at various stages of proximity to diagnosis in preHD are often underpowered to detect more subtle differences in social cognitive and executive function.^{358,364,675} Often observed is higher within-group variance compared to control samples, indicative of preserved function in many, and decline in function in some participants.³⁵⁴ Group-wise comparisons mask those who are more impaired by those earlier in the premanifest phase, or are closer to onset, but with preserved extramotor function. It underscores the importance for stratifying participants in this phase.³⁴⁶ Observation over periods of time can detect most subtle trends and increase power by reducing inter-individual variability through within-subject comparisons.^{603,707,708} There are no known longitudinal studies of social cognitive function in HD. This chapter specifically addresses whether changes in performance on social cognitive tasks and executive tests are detectable over time in PwHD.

8.1.1 Chapter aims

This chapter addresses **Aim 5: Hypotheses 5.1-5.3** as well as, **Hypotheses 6.3** and **7.2**. **Aim 5** is to evaluate social cognitive function as an early marker of cognitive dysfunction in HD, and as a candidate for extramotor disease onset. PwHD are expected to show significant impairments on a novel social cognitive battery (ACS-SP) relative to controls, with greater deficits emerging on tasks involving less naturalistic or context-poor stimuli. Social cognitive performance in PwHD is predicted to decline both with increasing CAG-associated disease progression estimates, and longitudinally over the course of the study. In this chapter, **Hypothesis 6.3** will be addressed longitudinally, controlling for speed of processing in a common executive inhibitory control function task. **Hypothesis 7.2** will further assess the discriminant validity of the RFI evaluating change-scores over time.

Together, these longitudinal analyses aim to clarify whether specific extramotor cognitive markers in social cognitive and executive domains can serve as sensitive indices of disease progression in HD, and to assess their utility for future diagnostic and staging criteria.

8.2 Methods

8.2.1 Study Design

This study had a longitudinal observational design. HD-Cognition participants were assessed at three timepoints (T1, T2, and T3), approximately 18 months apart, using repeated neuropsychological and behavioural assessments. Cross-sectional findings at baseline (T1) are reported in the previous chapter.

It was intended that n=50 participants would be recruited to each group (Manifest, Premanifest, and Controls), to ensure sufficient power for the three timepoints of the longitudinal design. With limited access to the patient population, these numbers were not reached. A number of participants joined the study later and have not yet reached their T3 follow-up appointment. Despite this, the available sample provides valuable data across the timepoints and results are interpreted with attention to sample size limitations and reductions in statistical power, particularly at T3. Mixed-effects models were used to maximise the use of available data while accounting for repeated measures and missingness.

8.2.2 Participants

Eligible participants in the HD-Cognition study were included in this study analysis. They fell into three groups at baseline: manHD, preHD, and controls (gene-negative family members or unrelated healthy controls). A flow-chart of longitudinal recruitment is shown in **Figure 23**. Reasons for attrition at follow-up appointments (T2/3) can be seen in the **Appendix 19**.

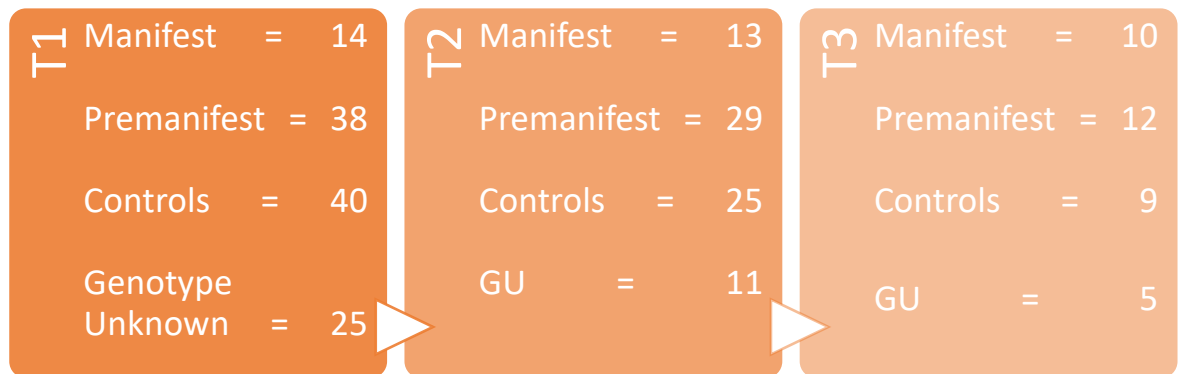
All Genotype Unknown participants were contacted in December 2024 to confirm their gene status. If known they were recategorized accordingly, otherwise there data were excluded from the analysis. They are included in recruitment flow chart for descriptive purposes.

8.2.3 Materials

Neuropsychological tests were the same as described under the HD-cognition study in the methods and cross-sectional analysis chapters. As in the cross-sectional analysis, the focus of this chapter is on tasks of social cognition and executive function to align with study aims and hypotheses.

HD-Cognition: Longitudinal recruitment

a)



b)

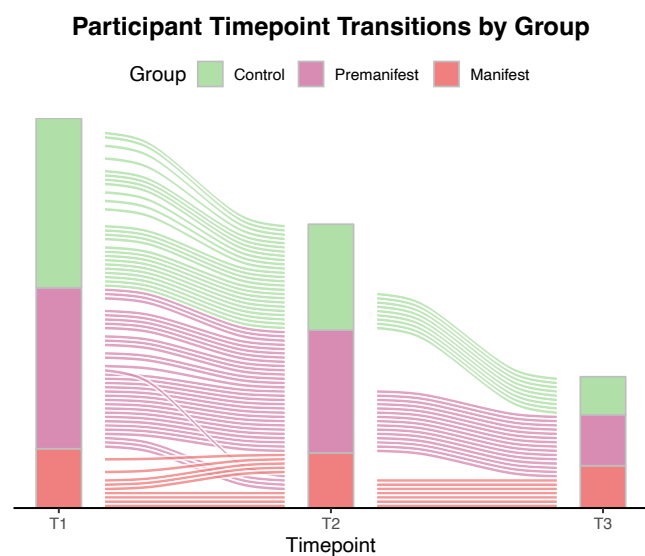


Figure 46: HD-Cognition longitudinal recruitment

a) Flow-chart for longitudinal study recruitment. n per group at each timepoint. **b)** Sankey plot to illustrate participant numbers through timepoints with premanifest to manifest transitions. (Premanifest-manifest, T1-T2: n=4; T2-T3: n=2)

8.2.4 Statistics

To assess longitudinal change in cognitive function across disease stages, linear mixed-effects models (LMMs) were used. Models included an interaction term to examine group effects over time, controlling for premorbid IQ (TOPF-FSIQ) and age (where not already standardised within z-scores). Group classification was based on baseline diagnosis (groupT1) and remained fixed across timepoints. To model CAG-Age-related disease progression continuously across the disease categories, additional LMMs modelled CAP100 and cognitive scores over time in PwHD with available CAG-repeat data (manifest and premanifest together). LMMs took the following structure:

Group×time LMM: ‘Cognitive test score’ ~ covariates + groupT1×timepoint + (1 id)

Disease progression LMM: ‘Cognitive test score’ ~ TOPF + timepoint + CAP100 + (1 id)

Marginal and conditional R^2 values were calculated to evaluate effect sizes for fixed effects alone and for fixed plus random effects, respectively. Values >0.3 were interpreted as large for marginal R^2 , and >0.6 as large for conditional R^2 .⁶²¹ Graphical representation of linear mixed effects models are based on estimated marginal means (EMMs) from model predictions, keeping covariates constant, at each timepoint.

Model-derived effects plots are used to illustrate the effect of estimated CAG-related disease progression in models with CAP100 scores as predictors in PwHD samples across timepoints. They plot the association between dependent variables/predictors and CAP100 scores, showing the 95% confidence interval around the predicted values. Scatter plots show the relationship between CAP100 and predictors as a function of diagnostic group at baseline (groupT1; manifest or premanifest) for descriptive purposes.

8.3 Results

8.3.1 Demographics at T2 & T3

Demographic data and sample comparisons at T2 and T3 are shown in **Appendix 20**. At T2, groups were matched on age, education, sex distribution and the interval from T1 to T2 (all $p > .05$). Welch two sample t-tests found no significant differences between the HD groups for CAG repeat length ($p = .2$), but the manHD group expectedly had significantly higher Disease Burden ($t(13) = -4$, $p = .003$), and CAP100 scores ($t(18) = -4$, $p < .001$).

At T3, groups were matched for sex ($p = .363$) and the interval from T2 to T3 ($p = .273$), but not for age ($F(2,28) = 4.55$, $p = .02$) or years of education ($F(2,28) = 5.83$, $p = .008$), where the manHD group were significantly older and less educated ($p < .05$). Welch two sample t-tests found no significant differences between the HD groups on CAG repeat length ($t(12) = -1$, $p = .2$), and again, the manHD group had significantly higher Disease Burden ($t(11) = -3$, $p = .01$), and CAP100 scores ($t(12) = -3$, $p = .008$).

8.3.2 Longitudinal cognition in premanifest & early HD

Descriptive statistics from the full HD-Cognition test battery at T2 and T3 are shown in **Appendix 20**. Questionnaire data are examined at baseline in the cross-sectional chapter and in **Chapter 9**. They are presented here for descriptive purposes.

Linear mixed-effects models examined cognitive performance over time. Model fixed effects predictors included group (Control, PreHD, ManHD), timepoint (T1, T2, T3), and group×timepoint interactions, with a random intercept for participant ID to account for individual-level variability over repeated measures. Covariates were also added—TOPF-FSIQ and age, where not included in z-score standardisation.

Collinearity is evident in some models between timepoint and group×timepoint interaction predictors, but the highest adjusted VIF remained below the threshold for concern. This suggests that values do not compromise the stability or interpretability of the parameter estimates, and so all predictors were retained in the models.

8.3.2.1 Social cognition: the RMET

The RMET model was based on 186 observations from 89 individuals, and included age as a covariate, z-scores are computed without it. The marginal R^2 was relatively high (0.52) compared to the conditional R^2 (0.85), indicating that over half of the variance in RMET performance was explained by fixed effects, with the remainder (33%) due to individual differences. TOPF-FSIQ was a significant predictor of RMET performance ($B(SE)=0.026(0.01)$, $p=.015$), indicating that individuals with higher estimated premorbid IQs tended to perform better on the RMET across all groups and timepoints. Age was also a significant predictor, with older participants showing lower RMET scores ($B(SE)= -0.021(0.01)$, $p=.022$), suggesting an age-related decline in social cognition across groups.

At baseline (T1), participants with manHD performed significantly worse than controls ($B(SE)= -1.98(0.33)$, $\beta= -1.52$, $p<.001$), almost 2SD below the normative population mean, controlling for age and TOPF. In contrast, preHD participants did not differ significantly from controls at baseline ($B= -0.215$, $p=.352$). In the control group, there was a trend-level improvement in RMET scores at T2 ($B=0.27$, $p=.072$), and a non-significant increase at T3 ($B=0.32$, $\beta=0.25$, $p=.144$), likely reflecting practice effects.

The manifest $\times$ T2 interaction effect was significant ($B(SE)= -0.736(0.28)$, $\beta= -0.56$, $p=.011$), indicating a further decline in RMET scores for manHD participants at T2. The premanifest $\times$ T2 interaction showed a non-significant negative trend ($B= -0.332$, $\beta= -0.25$, $p=.092$), attenuating the control level improvement. There were no significant interaction effects at T3 for either premanifest or manifest groups (both $p>.19$), suggesting no further decline beyond T2 relative to controls.

This model confirms that manHD is associated with significant social cognitive impairment which declines over time (age and premorbid IQ adjusted), particularly between T1 and T2. Trends are evident in premanifest participants, suggesting an absence of practice effects over time compared to controls, who improved (**Figure 47**).

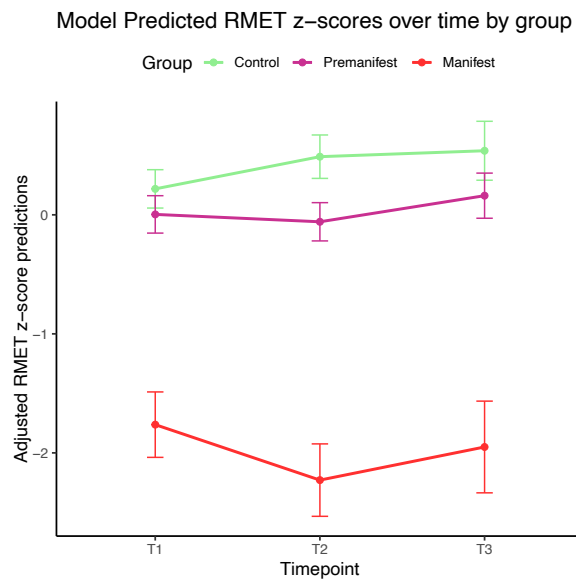


Figure 47: Model-predicted EMM plot for RMET scores over time by group, adjusted for TOPF-FSIQ and age.

8.3.2.2 Social cognition: affect recognition

The ACS-SP Affect recognition model was based on 172 observations from 86 individuals. The marginal R^2 was 0.42, indicating that 42% of the variance in affect naming performance was explained by fixed effects (premorbid IQ, group, time and group $\times$ timepoint), while the conditional R^2 was 0.73, suggesting substantial contribution of individual-level differences. TOPF-FSIQ was a significant predictor of affect recognition ($p=.024$).

The manHD group demonstrated markedly reduced performance compared to controls at baseline ($B(SE)=-2.19(0.35)$, $\beta=-1.84$, $p<.001$), performing over 2SD below the population mean. Premanifest participants, however, did not differ significantly ($B=-0.087$, $\beta=-0.07$, $p=.711$). Affect naming scores showed no significant change at T2 ($B=0.13$, $p=.496$) or T3 ($B=0.16$, $p=.547$), indicating a stable trajectory.

A significant group $\times$ timepoint interaction was observed for the premanifest group at T2 ($B(SE)=-0.655(0.25)$, $\beta=-0.55$, $p=.009$), suggesting a decline in affect naming performance at this timepoint compared to controls. A non-significant trend of similar magnitude was found at T3 ($B=-0.585$, $\beta=-0.49$, $p=.087$), whereby scores remained low relative to controls (**Figure 48**). No significant interaction effects were found in the manifest group across timepoints (all $p>.44$), indicating that the deficit in this group was present at baseline and remained relatively stable across time. Error bars in **Figure 48** demonstrate the increasing variability associated with decreasing sample size in the manifest group.

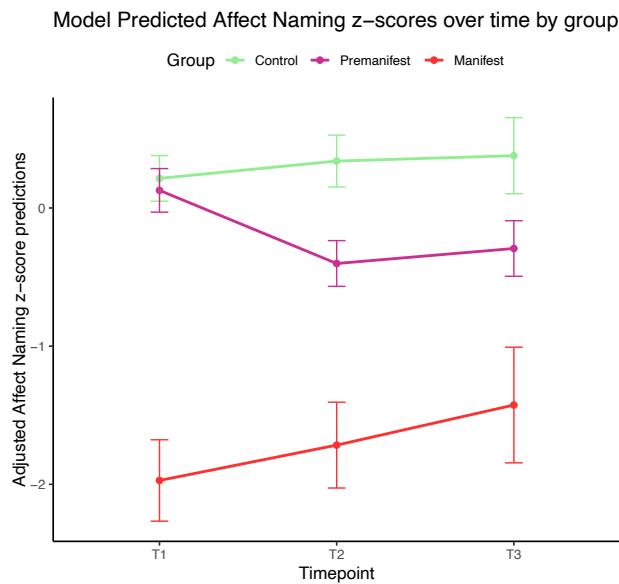


Figure 48: Model-predicted EMM plot for ACS-SP Affect Naming scores over time by group, adjusted for TOPF-FSIQ.

8.3.2.3 Social cognition: prosody-face matching

The Prosody-Face Matching model included 163 observations from 83 individuals. Fixed effects explained 41% of the variance in aural affect recognition and visual matching (marginal $R^2=0.41$), while the conditional R^2 was 0.74. Premorbid IQ approached significance as a predictor ($B(SE)=0.017(0.009)$, $p=.058$).

ManHD participants showed significant impairment compared to controls at baseline ($B(SE)= -2.09(0.36)$, $\beta= -1.78$, $p<.001$), performing $>2SDs$ below the mean. Premanifest group performance did not differ from controls ($p=.547$). There was no main effect of timepoint at T2 ($B=0.11$, $p=.543$), but a significant increase in performance was observed at T3 ($B=0.58$, $\beta=0.50$, $p=.028$), possibly indicating practice effects or non-random drop-out biasing scores upwards. No significant group $\times$ time interactions were observed (all $p>.7$), suggesting equivalent performance trajectories across time and groups **Figure 49**.

Overall, these results confirm a marked deficit in affective prosody-face matching in the manHD group, detectable at the earliest measured timepoint and persisting over subsequent assessments, which was not evident at the group level for premanifest participants when controlling for TOPF-FSIQ.

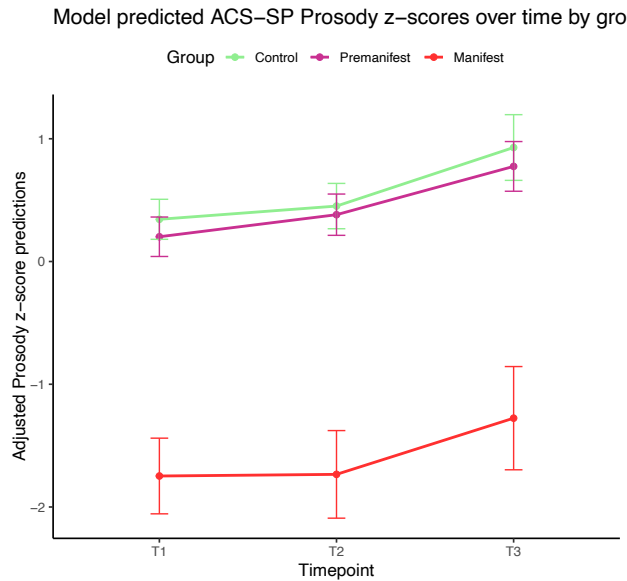


Figure 49: Model-predicted EMM plot for ACS-SP Prosody-Face matching subtest scores, holding TOPF-FSIQ constant.

8.3.2.4 Social cognition: Prosody-pair matching

A reduced numbers of manifest participants in particular had complete data for the ACS-SP Prosody-Pair matching subtest, limiting generalisability of these findings. However, it has multiple levels of enquiry per trial, requiring in-depth analysis of social situations and emotion perception, offering valuable insights into real-world social functioning. It also offers more contextual social information. The model was based on 144 observations from 78 individuals. Marginal R^2 indicating that fixed effects explained 38% of the variance, and conditional R^2 was 0.67. CR2 cluster-robust standard errors were applied to account for mild non-linearity in the residual structure, indicated by Rainbow testing ($p=.007$).

Main effects of group were significant, with manHD participants showing a large and statistically significant impairment relative to controls (CR2 SE applied: $B(SE) = -2.58(0.41)$, $\beta = -2.39$, $p_{CR2} < .001$). Although they tended to score lower, no significant difference was observed between premanifest and control participants ($B(SE) = -0.32(0.24)$, $\beta = -0.30$, $p_{CR2} = .184$). There were trend-level improvements at T2 ($B=0.28$, $p_{CR2} = .084$) and T3 ($B=0.60$, $p_{CR2} = .091$) which did not reach significance (**Figure 50**).

No significant group $\times$ timepoint interactions were found for manifest or premanifest groups (all $p_{CR2} > .05$), suggesting no further change beyond the baseline performance. Interpretation is limited by reduced numbers, non-random dropout and data MNAR in the manifest group however, indicated by increasing error bars shown in **Figure 50**.

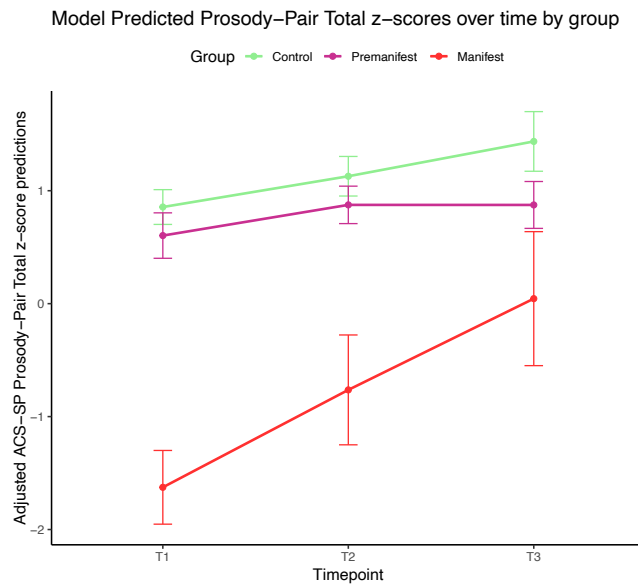


Figure 50: Model-predicted EMM plot ACS-SP Prosody-Pair matching subtest scores, holding TOPF-FSIQ constant.

8.3.2.5 Social cognition: Overall social perception battery

The Social Perception battery variable is an overall score, corrected for age, combining all ACS-SP battery subtests. The model included 144 observations from 78 individuals. Marginal R^2 was 0.43, while the conditional R^2 was 0.79. There was evidence of mild non-linearity by Rainbow test ($p=.03$), therefore CR2 robust standard errors were calculated, reported here.

Expectedly, manHD participants showed marked impairment on the social perception scale ($B(SE) = -2.20(0.31)$, $\beta = -1.88$, $p < .001$). Although scores trended lower, the premanifest group did not significantly differ from controls ($B = -0.20$, $p = .380$). Premorbid IQ was a significant positive predictor ($B = 0.026$, $p = .003$). There was no significant change at T2 ($B = 0.24$, $p = .130$), but performance increased at a trend level at T3 ($B = 0.61$, $p = .051$).

No interaction effects were statistically significant, indicating that score trajectory did not differ significantly between groups over time (all $p > .05$). Inspection of the plots (**Figure 51**) show premanifest participants remaining relatively stable compared to controls who improved over time, but this interaction effect did not reach significance ($B(SE) = -0.29(0.34)$, $\beta = -0.24$, $p = .4$).

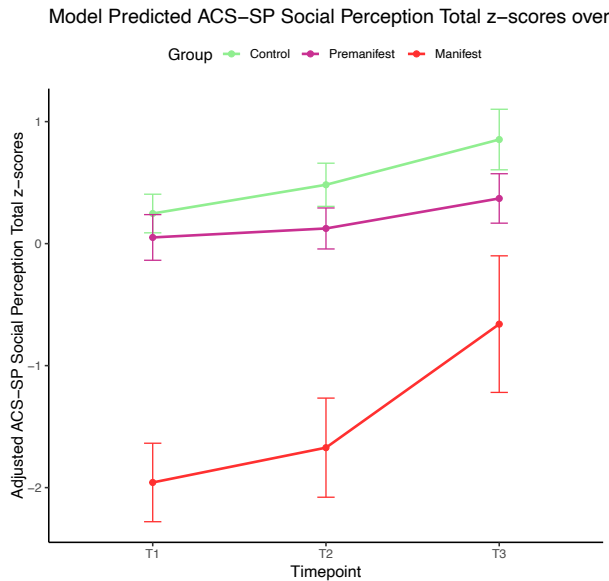


Figure 51: Model-predicted EMM plot ACS-SP Social Perception Battery Total subtest scores, holding TOPF-FSIQ constant.

8.3.3 Social cognitive performance with disease progression

To assess the effects of CAG-related estimates of disease progression on social cognitive tasks, CAP100 score was entered into LMMs examining test scores over time. These models only included PwHD with available CAG-repeat data (T1: n=41).

8.3.3.1 RMET

The LMMs including 92 observations from 41 PwHD revealed a significant effect of CAP100 on RMET scores, with higher estimated disease progression associated with poorer affective ToM/affect recognition, controlling for TOPF-FSIQ ($B(SE) = -0.026(0.01)$, $\beta = -0.51$, $p = .001$; see **Figure 52**). Timepoint and premorbid IQ were not significant predictors (all $p > .13$). The direction of the coefficients suggested marginal decline from T1 to T2 with small effect ($B(SE) = -0.254(0.17)$, $\beta = -0.19$, $p = .183$). The model explained 32% of the variance via fixed effects (Marginal $R^2 = .32$), and 84% when including subject-level random effects (Conditional $R^2 = .84$).

8.3.3.2 Affect Naming

The affect naming model included 85 observations from 40 PwHD. It revealed a significant effect of CAP100 on Affect Naming performance, with higher CAP100 associated with poorer scores after controlling for premorbid IQ ($B(SE) = -0.018(0.01)$, $\beta = -0.35$, $p = .029$; see **Figure 53**). The direction of the coefficients suggest small-to-moderate declines at T2 ($\beta = -0.27$, $p = .070$; **Figure 48**), and T3 ($p = .653$), but neither effect reached

significance, nor did TOPF-FSIQ ($p>.545$). Most variance (57%) in the model was attributable to inter-individual variability (Marginal $R^2=.13$; Conditional $R^2=.70$).

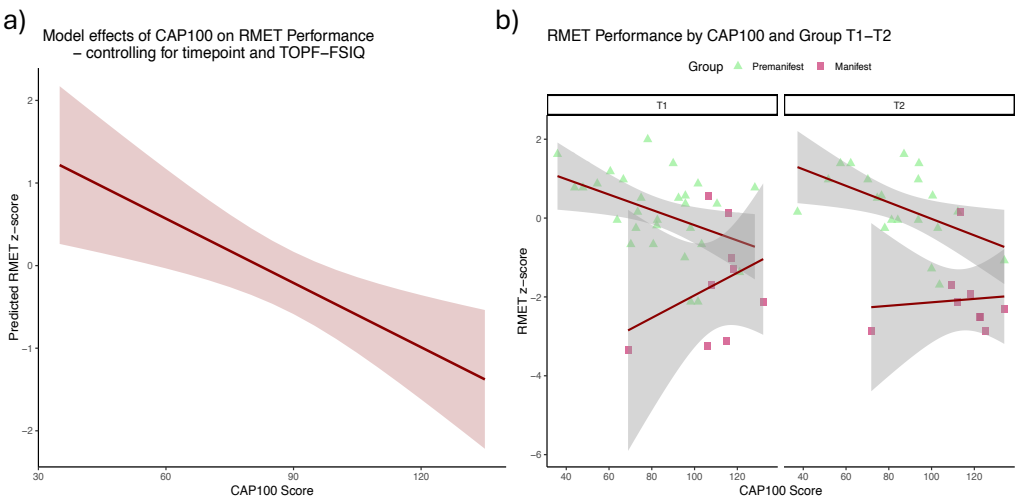


Figure 52: Model-derived effects plots: RMET.

NOTE: a) RMET performance shows strong relationship to CAP100 score, when controlling for time and TOPF-FSIQ, b) this shows a negative linear relationship in the premanifest group, but a more variable association in the manifest group at T1 and T2.

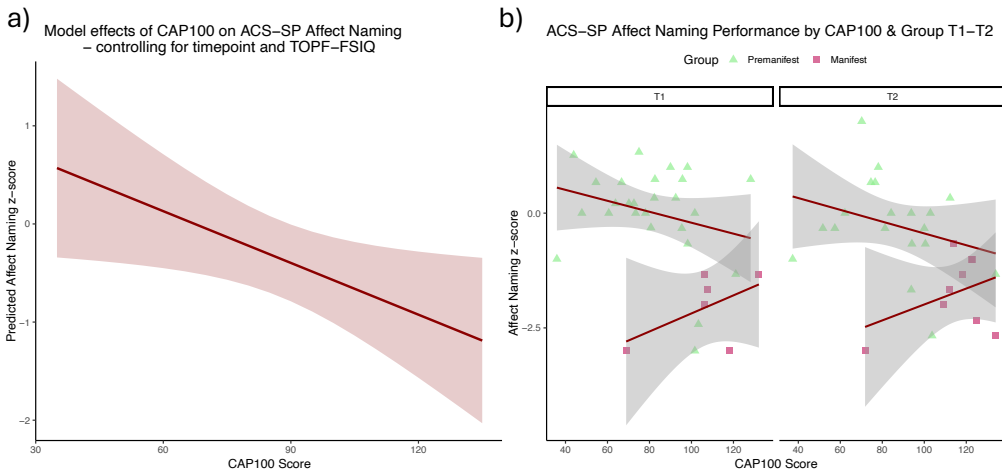


Figure 53: Model-derived effects plots: Affect Naming

a) ACS-SP Affect Naming performance shows strong relationship to CAP100 score, when controlling for timepoint and TOPF-FSIQ, b) this shows a negative linear relationship in the premanifest group, and a variable relationship in the manifest group at T1 and T2.

8.3.3.3 Prosody-Face matching

80 observations from 39 PwHD were included in the Prosody-Face matching model. A highly significant effect of CAP100 was shown, with higher estimated disease progression predicting poorer scores, after controlling for premorbid IQ ($B(SE)= -$

0.025(0.01), $\beta = -0.50$, $p = .001$; see **Figure 54**). Timepoint (T2) and TOPF-FSIQ were not significant predictors ($p > .14$), but the model showed significant improvements at T3, with large effect ($B(SE) = 0.782(0.21)$, $\beta = 0.62$, $p < .001$). The model explained 31% of the variance via fixed effects (R^2 : Marginal=0.31; Conditional=0.79).

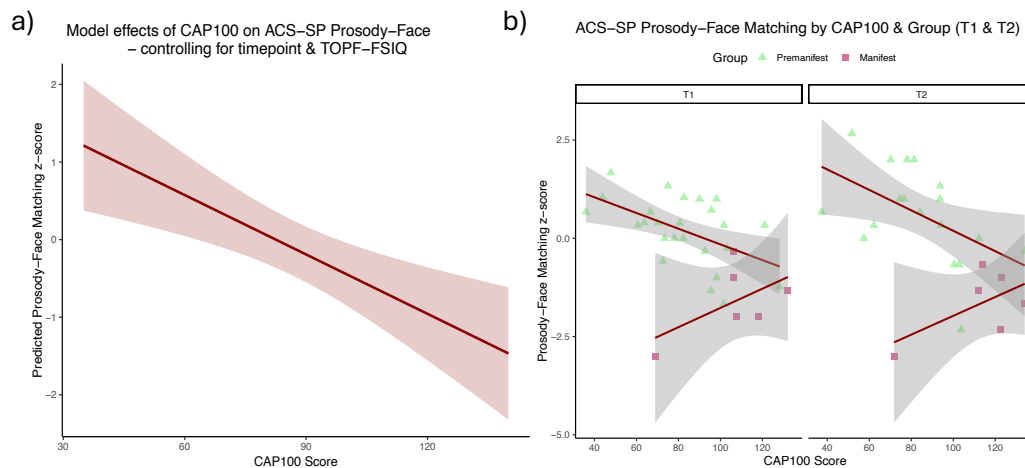


Figure 54: Model-derived effects plots: Prosody-Face Matching

a) ACS-SP Prosody-Face matching performance demonstrated an association with CAP100 score, when controlling for timepoint and TOPF-FSIQ, **b)** this shows a negative linear relationship in the premanifest group, and a variable relationship in the manifest group at T1 and T2.

8.3.3.4 ACS-SP Prosody-Pair matching

The Prosody-Pair LMM included 63 observations from 36 PwHD. The model explained 18% of the variance via fixed effects (R^2 : Marginal=.18; Conditional=.67). It identified a significant effect of timepoint for the more complex prosodic emotion recognition and visual-matching task (matching voice to pairs of people interacting). Performance improved significantly at T2 ($B(SE) = 0.62(0.23)$, $\beta = 0.55$, $p = .013$), with a trend toward improvement at T3 also ($B(SE) = 0.56(0.29)$, $\beta = 0.5$, $p = .059$). In contrast to other social cognition tasks, CAP100 was not a significant predictor of performance ($\beta = -0.22$, 95%CI: [-0.54, 0.11], $p = .200$). Neither did TOPF-FSIQ contribute significantly to the model ($\beta = 0.24$, $p = .157$).

Due to the relatively small number of observations the model may have been underpowered to detect small-to-moderate effects of CAP100 and premorbid IQ. Despite this, 95% CI are very wide and **Figure 55** indicates a flat relationship to CAP100 score, supporting an absence of true effect.

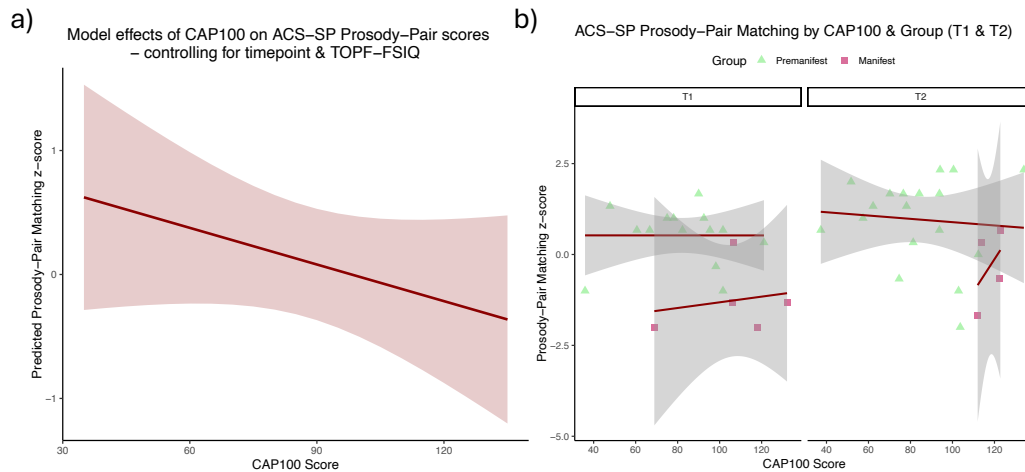


Figure 55: Model-derived effects plots: Prosody-Pair Matching.

a) ACS-SP Prosody-Pair matching association with CAP100 scores, when controlling for timepoint and TOPF-FSIQ, **b)** there is no discernible relationship in the premanifest or manifest groups.

8.3.4 Other variables of interest: longitudinal performance

Based on the cross-sectional findings in the previous chapter and to align with study aims, models for the longitudinal performance on verbal fluency tasks (FAS, semantic, RFI) and executive function tasks (working memory: digit span backwards (DSB); response inhibition: CWIT Inhibition time, contrast and error z-scores; speed of processing: SDMT) were constructed.

8.3.4.1 Verbal fluency: phonemic fluency

The model for phonemic verbal fluency (FAS) included 172 observations from 87 individuals. The marginal R^2 was 0.41, with a conditional R^2 of 0.84. A significant effect of premorbid IQ was observed ($B(SE) = 0.024(0.010)$, $p = .01$). Compared to controls, the manifest group showed significantly reduced performance ($B(SE) = -1.5(0.34)$, $p < .001$; **Figure 56a**). While main effects of timepoint were not significant, significant negative interactions were found between manifest participants and both T2 ($B(SE) = -0.85(0.29)$, $p = .004$) and T3 ($B(SE) = -0.90(0.41)$, $p = .032$). The premanifest group showed no significant interaction effects with timepoint (both $p > .7$). Together this shows decline in verbal fluency performance in the manifest group over time, whilst premanifest and control groups exhibit relative stability.

8.3.4.2 Verbal fluency: semantic fluency

The semantic fluency model included 180 observations from 88 individuals (R^2 : Marginal=0.39, Conditional=0.81). Higher TOPF-FSIQ was associated with improved performance ($B(SE)=0.022(0.01)$, $p=.038$) and the manifest group showed significant impairment compared to controls ($B(SE)= -1.83(0.38)$, $\beta= -1.35$, $p<.001$). Premanifest participants showed a relative reduction of just over half a SD, which approached significance ($B(SE)= -0.53(0.27)$, $\beta= -0.39$, $p=.051$; **Figure 56b**). T2 was associated with overall improvement ($B(SE)=0.56(0.17)$, $p=.001$). This improvement was attenuated by the interaction effect of premanifest group and T2, scores dropping almost half a SD between timepoints in this group, with a very large effect ($\beta= -1.96$), but it did not reach the threshold for significance ($B(SE)= -0.45(0.23)$, $p=.052$). The magnitude of scores were equivalent to an absence of practice effects, seen in the control group ($|B_{\text{control}}|0.56|\approx B_{\text{premanifest}}|-0.45|$). Scores in the manifest group, also fell across time points. This did not reach significance from T1-T2 ($p=.131$), and approached significance at T3 ($B(SE)= -0.815(0.46)$, $\beta= -1.79$, $p=.076$).

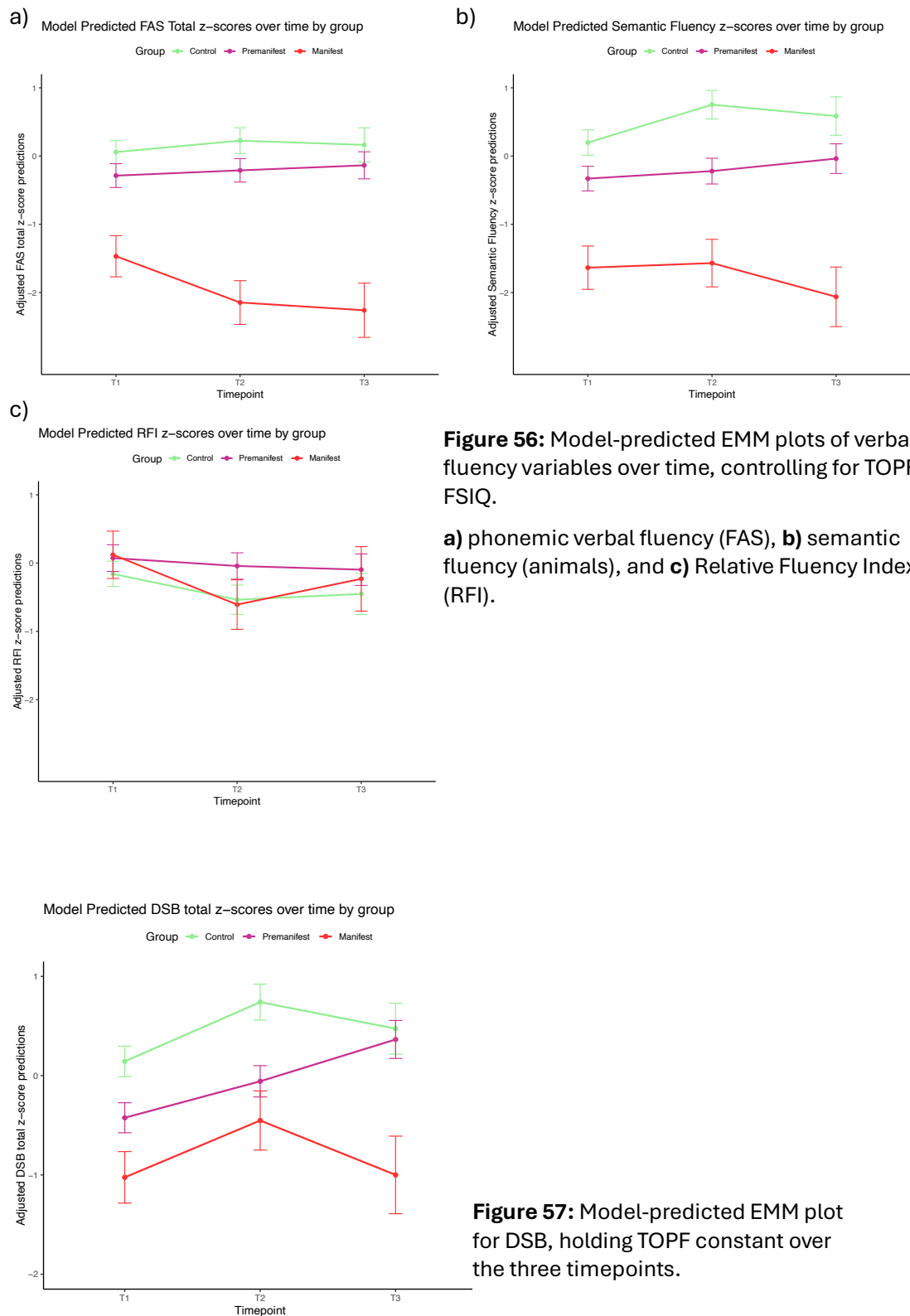
8.3.4.3 Verbal fluency: Relative Fluency Index (RFI)

The RFI model was based on 170 observations from 86 individuals. Fixed effects only explained 4% of the variation in RFI scores (Marginal $R^2=0.04$, Conditional $R^2=0.62$). TOPF-FSIQ did not improve the model, but was included as covariate for consistency. There were no significant effects for group, or the interaction of group $\times$ time (all $p>0.3$). The only significant effect was for timepoint (T1-T2), where scores decreased overall by 0.379 from T1 to T2 ($B(SE)= -0.38(0.19)$, $p=.048$), and a further 0.294 to T3, although not significantly ($p=.302$). This indicates a worse relative performance on the FAS task, or a larger relative improvement on semantic fluency over time, as is seen in manifest and control groups, particularly at T2 (**Figure 56b**). **Figure 56c** shows the relative ‘differential deficit’ fluency performance in each group does not differ significantly over time, remaining around the mean of 0 for all groups.

8.3.4.4 Working memory: Digit-span backwards

The DSB LMM included 188 observations from 91 individuals. Higher TOPF-FSIQ was associated with better performance ($p<.001$). Both the premanifest ($B(SE)= -0.57(0.22)$, $\beta= -0.47$, $p=.011$) and manifest groups ($B(SE)= -1.17(0.31)$, $\beta= -0.97$, $p<.001$) performed significantly worse than controls on this task, controlling for premorbid IQ estimates. Premanifest participants showed beta values almost perfectly intermediate to

control and manifest performance ($B_{\text{premanifest}} = -0.57; B_{\text{manifest}} = -1.17$; **Figure 57**). Performance improved from T1 to T2 ($B(\text{SE}) = 0.60(0.16)$, $p < .001$), with no significant group-by-time interactions, indicating all groups improved across timepoints, though not significantly at T3 ($p = .174$).



8.3.4.5 *Inhibition: CWIT Inhibition time*

An LMM for the CWIT Condition 3 subtest (Inhibition) included 182 observations from 87 individuals. This model explained very large proportions of fixed effects (52%) and total explained variance (91%; R^2 : Marginal=0.52, Conditional=0.91), indicating that both fixed predictors and individual-level random effects contributed substantially. Higher TOPF-FSIQ predicted better performance ($p=.010$). Both premanifest ($B(SE) = -0.66(0.24)$, $p=.007$) and manifest groups ($B(SE) = -2.35(0.35)$, $p<.001$) had significantly lower scores (**Figure 58a**). Performance improved at T2 ($B(SE)=0.27(0.12)$, $p=.031$) and T3 ($B(SE)=0.41(0.18)$, $p=.026$). Group-by-timepoint interactions revealed diminished gains, approaching significance for both PwHD groups at T2 ($p_{\text{premanifest}}=.054$, $p_{\text{manifest}}=.092$), and reaching significance at T3 ($T3_{\text{premanifest}}: \beta = -0.47, p=.008$; $T3_{\text{manifest}}: \beta = -0.72, p=.004$). This task does not control for general cognitive processing speed however.

The CWIT Inhibition-Naming contrast score, which does control for speed of processing (CWIT Naming subtask) giving a more representative measure of inhibitory control, shows no difference between the groups or any timepoint (all $p>.1$). TOPF-FSIQ is the only significant predictor, with a modest effect ($\beta=0.35$, $p=.002$). This suggests that the isolated executive inhibition performance on this task is accounted for by premorbid function at baseline. And, as described in the previous chapter, the uncorrected CWIT Inhibition score is largely confounded by reductions in speed of processing, as opposed to executive inhibition ability. **Figure 58a&b** highlight this discrepancy.

A model examining the z-score of error rates—failures to inhibit incorrect responses—found manifest participants performed significantly worse ($B(SE) = -1.19(0.42)$, $p=.010$), but premanifest participants did not differ significantly from controls ($p=.431$). Performance did not change significantly at T2 ($p=.126$) or T3 ($p=.831$), and no group-by-timepoint interactions emerged, indicating similar longitudinal error trajectories across groups (**Figure 58c**). Higher TOPF-FSIQ significantly predicted fewer errors ($p=.017$).

8.3.4.6 *Speed of processing: SDMT*

The SDMT test model included 178 observations across 86 participants. Conditional R^2 was 0.85, while the marginal R^2 was 0.45. ManHD participants performed significantly worse than controls, with a very large effect ($\beta = -2.02$, $p<.001$). Premanifest participants also performed significantly worse than controls across timepoints, nearly 1SD below the control group ($B(SE) = -0.94(0.36)$, $\beta = -0.49$, $p=.010$). Premorbid IQ was not a significant predictor of SDMT performance ($p=.86$).

There was a near uniform increase in SDMT scores across timepoints, which was significant at T2 ($\beta=0.30$, $p=.010$), and approached significance at T3 ($B(SE)=0.59(0.32)$, $\beta=0.31$, $p=.069$). Time-related increases were attenuated by trend level interaction effects of similar but opposite magnitude for both HD groups at T2 and T3 (see **Figure 59**), approaching significance for the premanifest $\times$ T2 interaction ($\beta= -0.28$, $p=.069$) and manifest $\times$ T3 interaction ($B(SE)= -1.09(0.61)$, $\beta= -0.57$, $p=.077$). The other two interaction terms did not reach significance ($p>.125$). These effects show controls improved over time, but that pre- and manHD HD groups did not benefit from practice effects, maintaining reduced baseline performance.

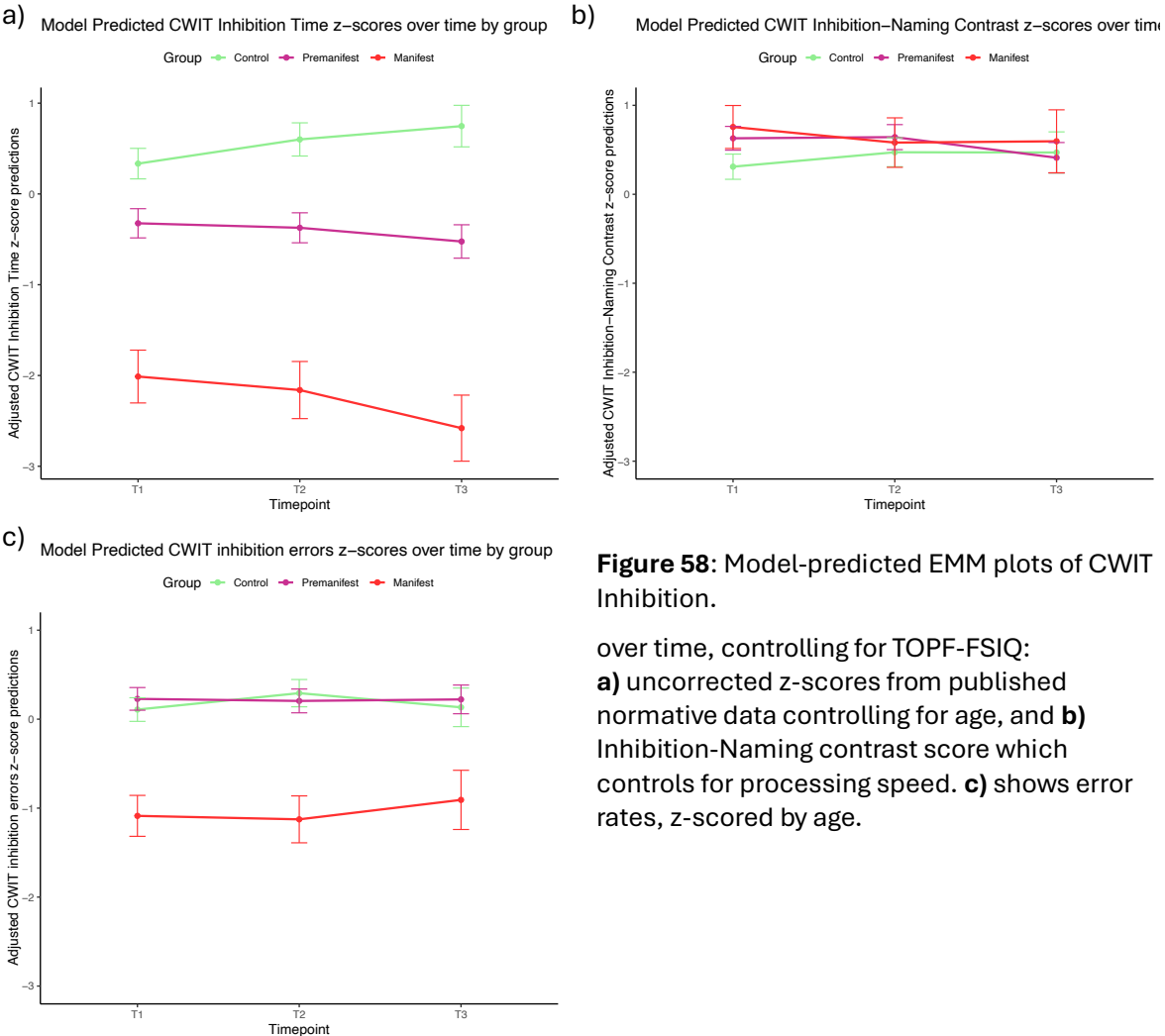


Figure 58: Model-predicted EMM plots of CWIT Inhibition.

over time, controlling for TOPF-FSIQ:
a) uncorrected z-scores from published normative data controlling for age, and **b)** Inhibition-Naming contrast score which controls for processing speed. **c)** shows error rates, z-scored by age.

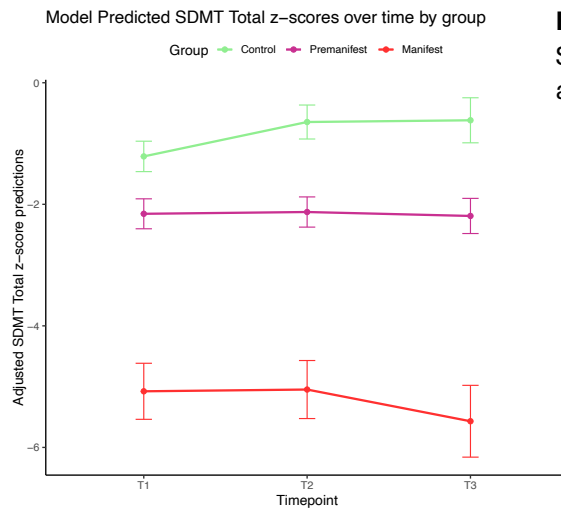


Figure 59: Model-predicted EMM plot for SDMT time to completion, z-scored by age and education, controlling for TOPF-FSIQ.

8.3.5 Executive function & disease progression

To assess the effect of estimated disease progression (CAP100) on cognitive scores, mixed-effects models were constructed for the outcome DVs of interest with TOPF-FSIQ as a covariate, and CAP100 and timepoint as predictor variables, as above. A summary of the disease progression mixed-effects model outcomes is shown in **Table 41**.

8.3.5.1 Verbal fluency

The phonemic verbal fluency model included 84 observations from 41 individuals. TOPF-FSIQ significantly predicted better verbal fluency ($B(SE)=0.034(0.02)$, $p=.045$), but there were no significant effects of timepoint (T2: $p=.791$; T3: $p=.65$) or CAP100 score ($p=.230$; **Figure 60a,b**).

The semantic fluency LMM included 87 observations from 40 individuals. Marginal R^2 was 0.24 and the conditional R^2 was 0.79, indicating that fixed effects accounted for a modest proportion of the variance, with considerable additional variance explained by individual-level differences (55%). CAP100 score was the only significant predictor ($B(SE)=-0.016(0.007)$, $\beta=-0.34$, $p=.025$), with disease progression estimates associated with poorer semantic fluency over time (**Figure 60c,d**). TOPF-FSIQ approached significance ($\beta=0.26$, $p=.071$),

The RFI model had 82 observations from 40 PwHD. Marginal R^2 was very small, 0.03, and the Conditional R^2 was 0.43, indicating that very little variance in restricted fluency was explained by the fixed effects (TOPF or CAP100). None of the predictors reached significance, including TOPF ($p=.57$), timepoint (T2: $p=.19$; T3: $p=.58$), or CAP100

score ($\beta=0.22$, $p=.16$), suggesting no reliable association with the relative fluency index in this sample, as hypothesised (**Figure 60e,f**).

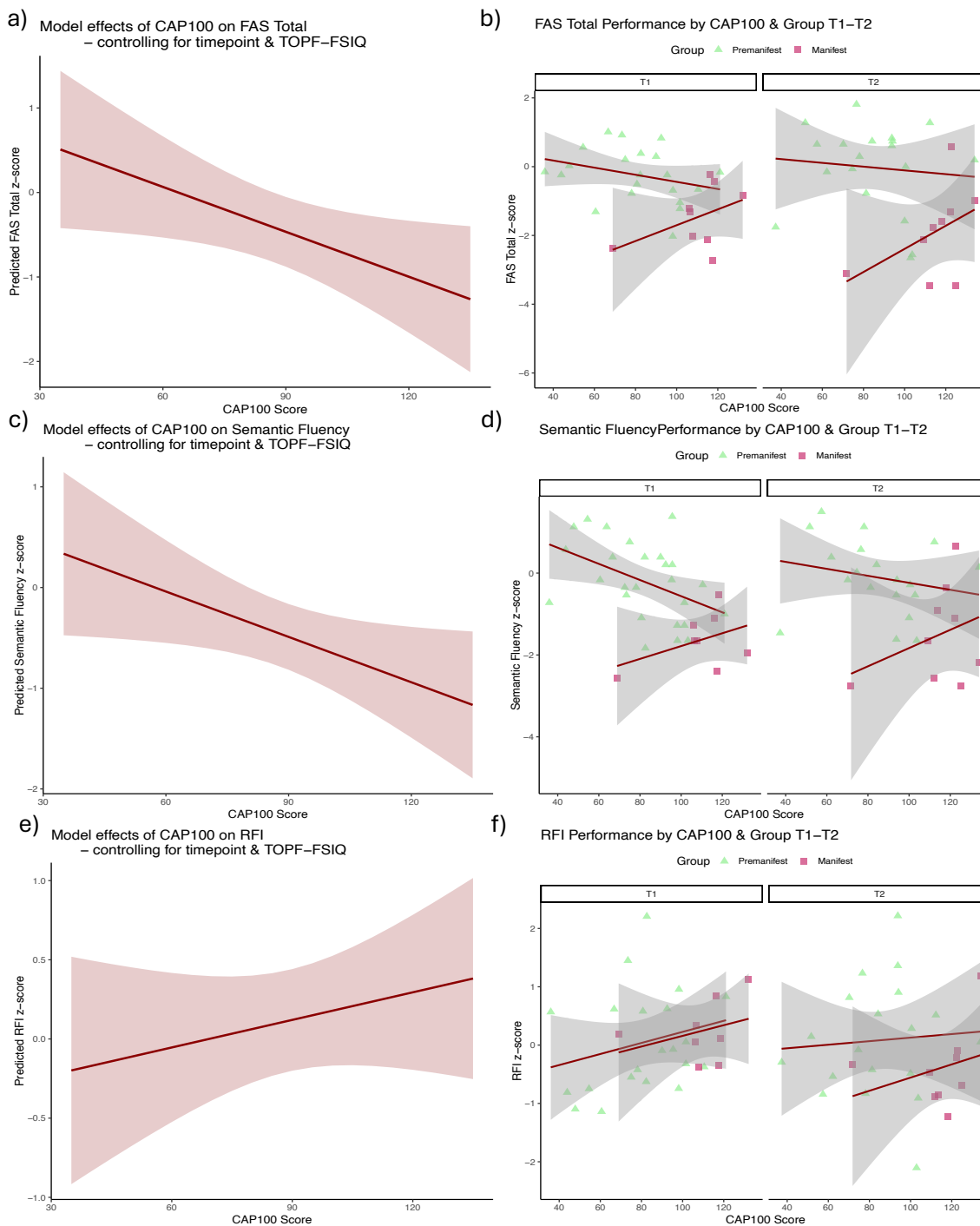


Figure 60: Model-derived effects plots: Verbal Fluency.

a,c,e) LMM model-derived effects plots and **b,d,f)** scatter-plots by group for T1 and T2 for verbal fluency scores. **a)** CAP100 was not a significant predictor in the FAS model. **c)** semantic fluency model-predicted values significantly declined with increasing CAP100, **d)** most highly related to premanifest group, at T1. **e,f)** There were no significant associations for the RFI. Bands depict 95%CI of the predicted variables, holding other predictors (timepoint, premorbid IQ) constant.

8.3.5.2 Working memory: Digit-span backwards

92 observations from 41 individuals were included in the DSB model. Higher premorbid IQ significantly predicted better working memory performance ($\beta=.30$, $p=.017$). Performance improved at both T2 ($B(SE)=0.453(0.15)$, $p=.004$) and T3 ($B(SE)=0.898(0.20)$, $p<.001$). Scores improved more than half a SD at T2 and 1.3SDs at T3, indicative of robust practice effects on this task (**Figure 57**). Nonetheless, CAP100 was negatively associated with performance over time ($\beta= -0.29$, $p=.035$; **Figure 61**), suggesting scores declined with increasing disease progression estimates over time.

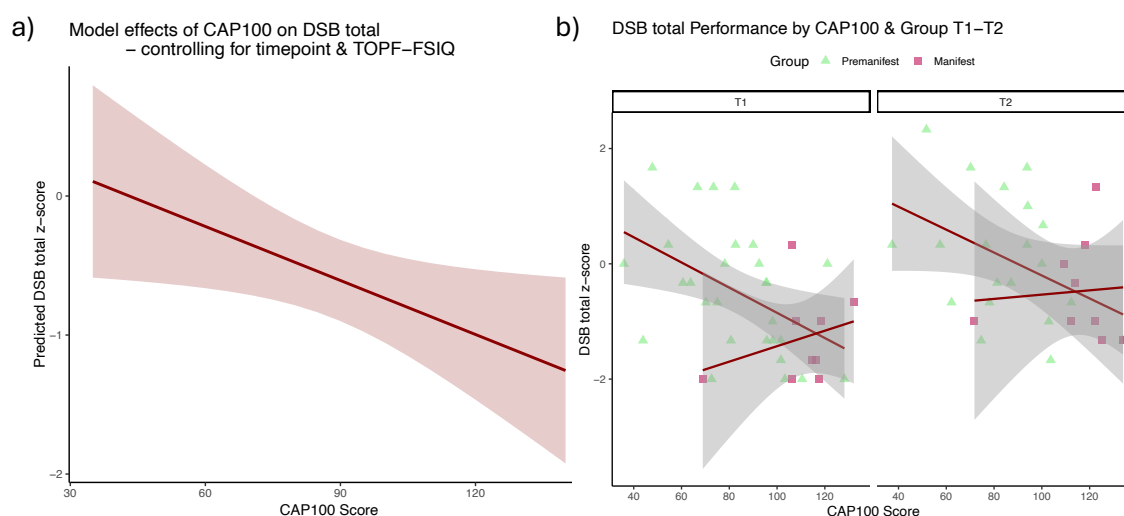


Figure 61: Model-derived effects plots: DSB.

a) model-derived effects plot, holding covariates constant, showing a significant negative association between CAP100 score and digit span performance, and **b)** scatterplots show the association with group membership and by timepoint.

8.3.5.3 Inhibition: CWIT Inhibition

The CWIT Inhibition models included 90 observations from 41 individuals. CAP100 score was a highly significant predictor of the uncorrected CWIT Inhibition time variable ($\beta= -0.40$, $p=.013$; **Figure 62a,b**), indicating that CAG-related estimates of disease progression are associated with slower performance (i.e.: lower z-scores). There was a trend toward improved performance with higher premorbid IQ ($\beta=0.26$, $p=.086$). There was no significant effect of timepoint (both $p>.4$). Fixed effects explained 27% of the variance in the model, with residual variance explaining an additional 61% (R^2 : Marginal=0.27, Conditional=0.88).

The CWIT Inhibition contrast score isolates executive inhibition from task performance factors (i.e.: speed of processing; Inhibition-Naming). In this model, there

was a trend toward improved performance with higher premorbid IQ ($\beta=0.29$, $p=.061$), controlling for age-matched baseline Colour Naming speed equal in magnitude to the effect in the uncorrected model ($\beta: 0.29\approx 0.26$). This reflects marginally improved inhibitory-control in those with higher premorbid IQ estimates, but not significantly so when controlling for CAP100 and time. Performance tended to decrease with successive timepoints but was only significant at T3 ($\beta= -0.44$, $p=.042$; T2: $\beta= -0.03$, $p=.869$), reflecting a decline in cognitive inhibition ability, above that of speed of processing that was not associated with estimated disease progression in the model (CAP100: $\beta=0.06$, $p=.698$; **Figure 62c,d**). Fixed effects only explained 9% of the model variance (R^2 : Marginal=0.09, Conditional=0.60).

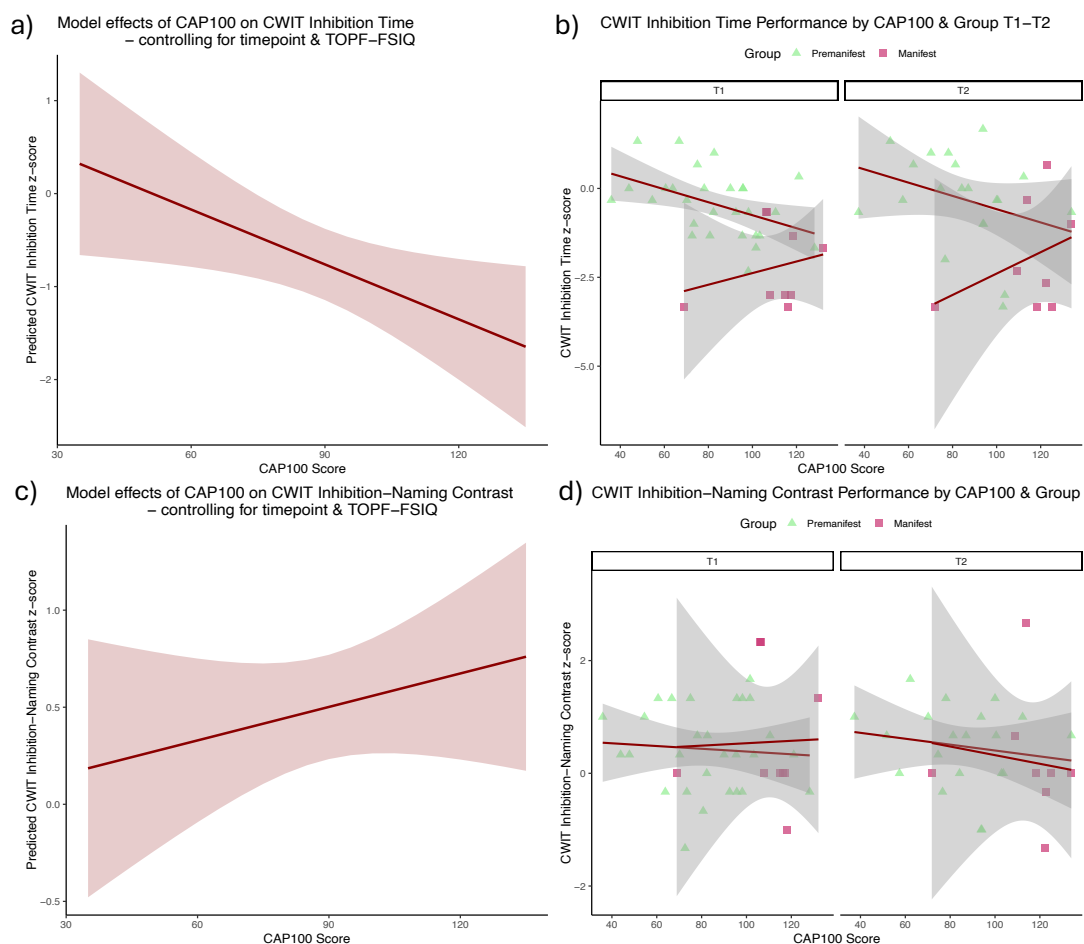


Figure 62: Model-derived effects plots: CWIT.

a,c) Model-derived effects plots for CWIT Inhibition models, showing strong negative association for, **a)** Inhibition time z-score and CAP100, but not for the speed-controlled contrast score, where it was not significant, **c)** **b,d)** scatterplots by time for T1 and T2 depicting strong negative association between CAP100 and uncorrected Inhibition score for premanifest, but not manifest PwHD. **d)** isolated executive Inhibition (speed-controlled contrast) is not associated to CAP100 score in either group at either timepoint.

8.3.5.4 Speed of processing: SDMT

SDMT performance showed considerable individual-level variability. The LMM (88 observations from 40 PwHD) demonstrated low marginal R^2 (0.19) and high conditional R^2 (0.84). Timepoint was not a significant predictor of SDMT performance (both $p > .3$). Higher CAP100 scores were associated with poorer performance ($\beta = -0.45$, $p = .007$), reflecting strong effects of CAP100 on processing speed (see **Figure 63**). Premorbid IQ was not a significant predictor in the model with very low effect ($\beta = .02$, $p = .876$), suggesting limited protective influence against disease-related decline in SDMT performance.

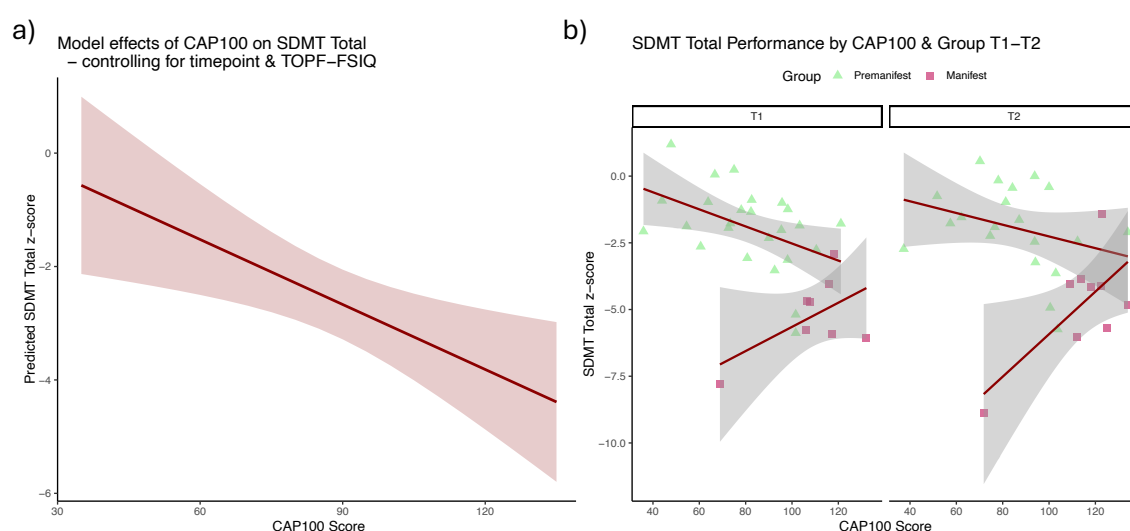


Figure 63: Model-derived effects plots: SDMT.

a) Model-derived effects plot for SDMT, showing strong negative association between predicted SDMT scores and CAP100. **b)** scatterplots by time depicting strong negative association between CAP100 and SDMT total time to completion scores for premanifest, but not manifest group. Bands show 95% confidence interval around the predicted values.

8.3.6 Summary of findings

Table 40 and **Table 41** summarise the mixed-effects model findings.

Table 40: Summary table for whole-group LMM results.

Social cognitive outcome variables								
Predictors	RMET	Affect Naming	Prosody-Face	Prosody-Pair	Social Perception: Total			
TOPF	x+	x+	.+	x+	x+			
Age	x+	NA	NA	NA	NA			
preHD								
manHD	x-	x-	x-	x-	x-			
T2	.+			.+				
T3			x+	.+	.+			
preHD_T2	.-	x-						
preHD_T3		.-						
manHD_T2	x-							
manHD_T3								
Other cognitive outcome variables								
Predictors	FAS	Semantic fluency	RFI	DSB	Inhibition: time	Inhibition: contrast	Inhibition: errors	SDMT
TOPF	x+	x+		x+	x+	x+	x+	
preHD		.-		x-	x-			x-
manHD	x-	x-		x-	x-		x-	x-
T2		x+	x-	x+	x+			x+
T3				.+	x+			x+
preHD_T2		.-			.-			.-
preHD_T3					.-			
manHD_T2	x-	.-			x-			
manHD_T3	x-	.-			x-			.-

For the LMM: 'cognitive test score' ~ TOPF + groupT1xtimepoint + (1 | id)

x = significant main effect of indicated predictor

. = trend level effect

+ / - = direction of the effect

Table 41: Summary table for CAP100 LMM results. PwHD group only

	Social cognitive outcome variables			
Predictors	RMET	Affect naming	Prosody matching	Prosody Pair
TOPF				. +
T2				x +
T3				
CAP100	x -	x -	x -	

b)	Other cognitive outcome variables						
Predictors	FAS	Semantic fluency	RFI	DSB	Inhibition: time	Inhibition: contrast	SDMT
TOPF	x +			x +	. +	. +	
T2				x +			
T3				x +		x -	
CAP100		x -		x -	x -		x -

For the LMM: 'cognitive test score' ~ TOPF + timepoint + CAP100 + (1 | id)

x = significant main effect of indicated predictor

. = trend level effect

+ / - = direction of the effect

8.4 Discussion

The findings of the longitudinal study build on the cross-sectional findings in the previous chapter, contributing to the understanding of cognitive trajectories in premanifest and early manifest HD. Longitudinal studies of cognition in HD have typically assessed executive function and psychomotor processing speed, but without distinction for the effects of one on task performance.^{228,262,316,391} Longitudinal studies of social cognitive function are lacking, and this may be the first reporting assessments of multiple domains.^{358,675} The executive and speed of processing results largely align with findings in the TRACK-HD and PREDICT-HD studies.^{255,261,345,709} I have extended these to examine task-validity and multidomain assessment of social cognition using mixed-effects modelling approach. Progressive decline is demonstrated across tasks, for manifest but also premanifest PwHD, aligning with existing evidence of early and marked vulnerability of frontostriatal circuitry in HD.

8.4.1 Longitudinal cognitive change

8.4.1.1 Social cognitive function

Studies assessing change in domains of social cognition are lacking in HD, with no known studies presenting multidomain data over time. In this study, although manifest participants show global social cognitive impairments, the ACS-SP prosody subtests do not show the discriminatory power in the premanifest group, mirroring the findings from the cross-sectional study. The premanifest group did not differ from controls at baseline on tasks of emotion recognition and ToM. However, scores decreased longitudinally. The RMET and Affect Naming subtasks were most sensitive, controlling for premorbid IQ estimates, distinguishing premanifest participants from controls in the models, but only at the trend level for the RMET. This underscores the importance of longitudinal assessment in the premanifest phase for identifying signals that may otherwise be missed, supporting their use as early cognitive biomarkers.

However, it should be noted that these effects were mostly shown by premanifest participants not benefitting from the practice effects demonstrated by the control group in successive sessions as opposed to absolute decline from baseline. Standardised regression formulae have proven sensitive to such patterns in MCI⁷¹⁰ and could be applied to highlight subtle changes not apparent at face-value in the premanifest phase where declines are relatively small point-to-point and can be occluded by practice effects. These findings are consistent with cross-sectional studies in premanifest PwHD up to 15

year prior to diagnosis, and which were associated with estimated proximity to diagnosis.^{356,681,711}

8.4.1.1.1 *The ACS-SP battery*

Aim 5 of this thesis was to assess the validity of the cohesive battery of social cognitive tests within the ACS-SP for use in HD, hypothesising that tasks would distinguish premanifest and control participants at baseline and longitudinally over the study period (36 months average), as well as show an association with disease progression. Subtests detected large deficits across all domains for manifest PwHD.

The significant decline in premanifest participants at T2 longitudinally in the group analysis, in addition to CAP100 disease progression estimates suggests that the ACS-SP Affect Naming task is sensitive to the emergence of subtle affective processing difficulties prior to clinical diagnosis, substantiating **Hypothesis 5.3a**. CAP100 was significantly associated with poorer performance on all social cognitive tasks, except the Prosody-Pair scale (see **Table 41a**), reinforcing the ACS-SP's suitability for the assessment of premanifest social cognitive function in HD. Overall it highlights the viability of social cognitive tests as early markers of extramotor onset in HD.^{228,354,356,358,375}

Decreased number of observations for the Prosody-Pair Matching subtest limits interpretability. However, figures plotting the model parameters show flat curves on this task. Together these findings further support **Hypothesis 5.2**, discussed in the last chapter, that performance reflects compensatory mechanisms afforded by multimodal contextual cues in this task⁶⁹⁶ and that affective ToM and emotion recognition tasks appear more vulnerable to early disruption (RMET and ACS-SP subtasks). Replication with larger samples is warranted to confirm the stability and timing of these effects using the ACS-SP, and fully evaluate the Prosody-Pair scale as a more naturalistic ecologically valid assessment of social cognition.

8.4.1.2 *Executive function, processing speed & working memory*

Large studies in preHD have demonstrated longitudinal decline across cognitive domains as secondary outcomes, primarily psychomotor-processing speed and speeded executive function tasks.^{255,298,316,390,712} Concordant with these, the current study shows large effects for reduction in speeded task performance at baseline, with trend level declines also demonstrated over time (particularly at T2) for the CWIT Inhibition and SDMT tasks. By tapping cognitive slowing, these tasks offer the most sensitivity to early change in HD, hence the inclusion of the SDMT in the HD research staging system (HD-ISS).²⁵⁶ But again it is highlighted here that the CWIT Inhibition task reflects speed of processing

deficits, not necessarily executive function. Scores isolating the inhibition component do not decline over time, nor do the increased error rates observed at baseline in manHD, further supporting **Hypothesis 6.3**. The CWIT Inhibition-Naming contrast scores were only predicted by premorbid IQ, not disease progression or timepoint, indexing the independence of the latent executive constructs of this task from disease-related slowing, which are associated with general intelligence and confer protective effects to executive function.⁷¹³⁻⁷¹⁵

Semantic fluency performance was also lower in preHD, approaching significance both at baseline and for longitudinal decline over time (T2), but was highly negatively associated with CAP100 scores. This is in contrast to the phonemic verbal fluency task, typically affected in ALSci, which showed no effects in premanifest participants, though declined steadily with strong effects in the manifest group, more than semantic fluency. Contrasting to ALSci, where phonemic verbal fluency deficits are specifically apparent^{16,118}, the HD-Cognition study, and the HD cognitive literature, show non-specific fluency deficits, evidenced here by RFI mean scores equivalent to controls, with semantic fluency the most impaired in the current study. Fluency scores decreased over time in the manifest group, and marginally in the premanifest group for semantic fluency. Preserved error monitoring and FAS performance in preHD suggests that subtle deficits in energisation and dysfunction of the striatal-superior-medial circuit begin to emerge in premanifest stages, with more deficits emerging closer to clinical diagnosis also involving dorsolateral-striatal circuits, evidenced by significant manHD decline across time for phonemic and semantic fluency tasks.^{332,528} There was a minor divergence on verbal fluency tasks, whereby semantic fluency was associated with CAP100 disease progression, trending in the premanifest group longitudinally, but phonemic fluency was not. Semantic fluency therefore evidences specificity to progression. However relative differences were small in degree, as the RFI did not differ by group, nor was it associated with disease progression, indicating divergent validity from its use in ALS, and supporting **Hypothesis 7.2**.

8.4.2 Cognitive associations to disease progression

Despite the variability in symptom presentation indicated in the scatterplots, CAP100 shows strong predictive utility as a stratification and prognostic tool here. Concordant with the literature, findings support its continued use in HD clinical research.^{255,256,315,317,346,391,716-718} Findings also partially substantiate both aspects of

Hypothesis 5.3, whereby CAP100 scores, estimates of CAG-associated disease progression, are negatively associated with the three primary social cognitive tasks (RMET, Affect Naming, Prosody-Face Matching), as well as semantic fluency, working memory (DSB), and processing speed (SDMT and CWIT Inhibition). These tasks may prove most sensitive for examination of extramotor function across premanifest and early manifest HD.^{255,256,346,391}

However, when controlling for processing speed, this relationship is attenuated, or disappears entirely, as with the CWIT Inhibition contrast score. This emphasises speeded tasks as particularly sensitive in early HD, but also the need to control for speed, should the evaluation of cognitive constructs be the research goal, as it is here. For clinical trials, reduced cognitive speed can be leveraged as a cognitive biomarker for HD, should reliable tests be used. CAP100's predictive strength across cognitive domains reinforces its utility as a reliable stratification variable for cohort studies and clinical trials for cognitive as well as motor function^{223,315,318}, consistent with longitudinal studies in HD.⁷¹² However other studies find a weaker relationship between CAP scores and cognitive and motor function, which can diverge independent of CAG-associated factors.^{389,390} These findings are consistent with stochastic somatic expansion in striatal MSNs differentially affecting striatal nuclei, and related to but not determined by peripheral or basal CAG-repeat length.^{271,719} In lieu of reliable markers of somatic expansion and genetic pathology in striatal neurons, clinical trajectories and slopes may offer better prognostic information than current parametric survival modelling techniques.^{314,319,389,390,453}

CAP100 score was often associated with declining cognitive scores, particularly in the premanifest group. Variability in cognitive function at and around motor symptom onset evident in the CAP100 analysis scatter plots is concordant with findings of three phenotypic clusters based cognitive and behavioural impairment at motor-diagnosis: primarily cognitively impaired, behaviourally impaired, and cognitively preserved.³⁰¹

8.4.2.1 Practice effects

Practice effects were evident across groups on most cognitive tasks despite exaggerated effects in the manifest group, likely by attrition and non-random dropout. This is apparent on model-predicted EMM plots show increasing scores across timepoints on most tasks, particularly for controls. Common across shorter intervals^{100,169,720}, less of an effect would be expected across the 18-20 month testing intervals. However, practice effects can be apparent even at intervals of up to 2 years.⁵³⁷ This was most prominent on

the working memory task (DSB) and SDMT. In general, working memory tasks are vulnerable to learning effects which may mask decline, and for this reason, processing speed is often more sensitive.⁴⁶⁹ Speed of processing (SDMT) was significantly reduced in both pre- and manHD, but the substantial practice effects which occur on the SDMT mean this test is unlikely to be reliable on repeat testing, without proper controls. This is cause for concern, as the SDMT is the extramotor clinical sign landmark in the HD-ISS research classification criteria²⁵⁶, and is used universally as a marker of cognitive dysfunction in HD (and other conditions), including as surrogate endpoint in clinical trials. Trial runners and clinical researchers should use validated alternate forms, as are available in the RBANS, for example.^{343,721} Evidence of such strong practice effects has implications for large cohort studies such as Enroll-HD which does not use alternative test versions^{538,539}, and does not offer indices to identify significant change.⁷²²

Here attenuated practice effects on are common across tasks in the premanifest group, including the SDMT, where scores did not improve at the same rate as controls. While absence of improvement does not constitute decline, absence or decreased magnitude of practice effects are associated with prognosis and current status in an MCI population.⁷²³ Practice effects (or lack thereof) can be leveraged as markers of decline.⁵³⁶ However, compounded with the protracted emergence of extramotor symptoms that occurs for many PwHD in the premanifest phase, practice effects make evaluation of cognitive function over time difficult in HD.⁷¹² In addition to regression-based methods⁷¹⁰, indices of reliable change, discussed in previous chapters, are also suited to these applications, controlling for practice effects and evaluating meaningful decline in HD research and clinical practice.⁷²³⁻⁷²⁵ There are many large cohort studies well placed to address this.

Variability is seen in manHD cognitive scores relative to CAP100 across cognitive tasks (see scatter plots), which is more uniformly negative and linear in premanifest participants. Further evidence of this is the significantly lowered SDMT scores in both PwHD groups, where attenuation of practice effect is also evident across timepoints. CAP100 scores are significantly negatively associated with semantic fluency, uncorrected inhibition time and processing speed (SDMT), and show a trend level effect for working memory (DSB) in PwHD (see **Table 41b**).

8.4.2.2 Error rates

Error rates were only increased in the manHD group, and were stable over time. Count data may be less sensitive to change, and as noted, practice effects were

significant on tests in which errors were secondary outcomes (SDMT, CWIT Inhibition), contributing to this effect. Tasks specifically tapping dorsolateral-striatal circuit function with primary outcomes of task setting and error monitoring such as Go/No-go tests are likely to show larger effects of inhibitory and attentional control over time.^{332,726}

8.4.2.3 Covariate effects

TOPF-FSIQ was a significant predictor of performance on all tasks except speed of processing, where group effects were highest. This suggests that individuals with higher scores tended to perform better across timepoints and groups, including for social cognitive subtests. It is most apparent on tasks with high complexity, but effects are diminished on the speed of processing task (SDMT) where performance across groups, and in subgroup analyses, is largely CAG and age dependent. It also indicates that between-group differences in premorbid IQ at baseline contribute significantly to task performance, and should be addressed. Controlling for this mitigates the confounding effects of variability due to demographic differences. This is demonstrated in the PwHD-only analysis for CAP100, where TOPF-FSIQ did not predict most scores, and disease-progression (CAP100) contributed more to explanatory power. Despite this, premorbid IQ was a significant factor in the phonemic verbal fluency and working memory models (and approached significance for semantic fluency), even when accounting for disease-related factors, suggesting a strong association to premorbid ability. Pertinent to the hypotheses of this thesis, TOPF-FSIQ contributed almost equally sized standardised-beta coefficients (β) to the CWIT Inhibition time and contrast subtest scores. Together with the fact that TOPF was not associated with SDMT scores (speed of processing), it highlights the relative independence of speed of processing ability from IQ estimates.

Most models and comparisons presented in this thesis control for TOPF-FSIQ premorbid IQ estimates due to significant group differences. The TOPF-UK is highly correlated with education, and is standardised based on age- and education-stratified normative data. Including it as a demographic covariate endeavours to highlight specific cognitive profiles and deficits above general impairment, but it may dampen true effects of between-group and inter-individual differences, reducing explanatory degrees of freedom where it is less relevant, and potentially increasing Type II error for these general effects.

TOPF-FSIQ was a significant predictor in all social cognitive tasks. It also builds on the cross-sectional findings which showed associations with verbal ability on emotion recognition tasks that were significantly smaller for the prosody subtest. This suggests

crystalised verbal intelligence plays a role in social cognitive function, which PwHD participants are less reliant on in the multi-modal tasks. Age was also a significant predictor in the RMET model here, further emphasising the importance of controlling for premorbid IQ estimates and age in the assessment of social cognition. This is pertinent, as the RMET does not have published education- and/or age-stratified normative data in English.⁷²⁷

8.4.3 Study-specific limitations

Control and premanifest participants who agreed to T2 and T3 were slightly younger and had higher education, whereas the manHD participants were representative across time.

Attrition changes group demographics over time in the HD-Cognition study. Whilst well matched at baseline and T2, the small manHD group are significantly older and less educated at T3, and show progression on clinical scales (UHDRS). Follow-up timepoints are also likely effected by non-random drop out of more impaired individuals (attrition bias) indicated by trends for increasing scores on most of the tasks, for the manifest group in particular. This may have skewed results of practice effects, which were large on the SDMT and DSB. However the control and premanifest groups remained representative across timepoints.

Attrition limits generalisability on the ACS-SP Prosody Pair and Social Perception totals in particular, which had small numbers due to the late introduction of these tests. The longitudinal study has many of the same task and missing data limitations as outlined in the previous chapter. Error at T3 was higher, particularly in the manHD group, and trends of similar magnitude to previous timepoints were not statistically significant. Many of the models returned non-significant trends with medium-to-large beta-coefficients in preHD also, which may have proven significant effects with increased power (**Table 40**). Linear mixed-effects modelling over multiple timepoints increases statistical power, which tackle this limitation.

Across most tests, there is one manHD participant who has a low CAP100 score, skewing the regressions for the manifest group and weakening the strength of the effects of CAP100 overall (for example, see **Figure 54b**). This is genuine case, and was not considered a highly influential point in the models, but serves to illustrate the limited predictive ability of the CAP100 score, evidencing the heterogeneity in CAG-related effects on clinical-motor diagnosis. Somatic expansions undermine CAP-based

predictions as drivers of variability not captured by CAG-repeat associated predictions from peripheral tissues.^{271,287,451} Thus, while CAP100 is a valuable stratifier, it should be complemented by direct clinical and cognitive measures in prognostic modelling, such as the PIN_{HD}, which integrates SDMT and motor scores to CAG-associated predictions.³¹⁹ HD-Cognition focuses on neuropsychological profile of PwHD. Data on motor symptoms and imaging biomarkers are not captured directly, which precludes the use of these indices and limits staging according to the HD-ISS²⁵⁶ and the manifest disease.

8.5 Conclusions

These findings support longitudinal investigations of extramotor function at the group level in HD, highlighting areas of investigation likely to produce effects in preHD. CAP100 consistently predicted decline across tasks, though its predictive limitations highlight the importance of incorporating multimodal markers (e.g.: imaging, cognitive composites) into progression models.

Together with the cross-sectional findings of the last chapter, there is strong evidence for impairments of cognitive processing speed, which declines over time and with CAG-associated estimates of disease progression, but that a speed-free executive dysfunction is a less significant marker of change in this PwHD sample. Cognitive slowing, not inhibitory control, drives the group differences. Executive inhibitory control may be preserved longer than acknowledged in the literature. This nuance is critical for interpretation of the neuropsychological profile in HD.

Speed of processing and working memory tasks are most sensitive in preHD at baseline, whereas affective ToM/Affective recognition, semantic fluency and speed of processing tasks are the best candidates for monitoring change over time and with CAG-associated disease progression, supporting their sensitivity as early cognitive markers for HD.

9 Chapter 9:

The essence of apathy in neurodegeneration: profiles and cognitive correlates

9.1 Introduction

Apathy is the most common behavioural symptom across neurodegenerative diseases of the motor system, as well as purely cognitive disorders^{381,728} and a significant clinical feature of ALS and HD.^{152,158} Psychiatric symptom trajectories have been explored previously in HD, with increases evident across the prodromal phase, but apathy was not included.³⁷⁸ Decline across manifest disease has been demonstrated.^{729,730} The longitudinal progression and development of apathy over time is less represented in the HD literature. Most descriptions are cross-sectional, and do not directly observe the relationship between domain-specific apathy and cognitive function.

9.1.1 *Apathy & cognition*

Apathy has been shown to correlate with performance on a number of cognitive tasks.^{387,731-733} Apathy, executive functions and social cognitive dysfunction have shared neuronal networks.^{349,373,385,734} Damage to the superior-medial fronto-striatal circuits which include the ACC and DLPFC disrupts cognitive control energisation, and processes underpinning rapid information search and selection.^{141,332} This manifests as poorer performance on timed executive tasks, such as phonemic verbal fluency, and behaviourally as increased Initiation apathy. The association between ECAS verbal fluency scores and Initiation apathy have demonstrated this overlap in pwALS, with higher apathy scores associated with lower phonemic verbal fluency.⁷³³ These factors may be related to one another through shared neural mechanisms governing their function, but also may have a behavioural effect in neuropsychological assessment and screening, whereby pwALS experiencing initiation apathy, are less able to initiate and perform tasks requiring effortful engagement. The precise constructs that are affected are poorly characterised due to the multidomain requirements of these tasks.

A recent study by Hendel et al.⁵⁰⁰, reports executive function and affect recognition are related to overall apathy in HD, but not when controlling for motor dysfunction. In contrast, Kempnich et al.(2018)⁷³⁵ found that emotion recognition performance on the TASIT was associated with FrSBe Apathy, independent of motor dysfunction. Both treat apathy as a unitary construct, without differentiating sub-types.

Depressive symptoms as well as CAG repeat length have demonstrated relationship to cognitive and apathy scores in preHD.^{500,736} It is therefore essential to control for depressive symptoms to avoid confounding effects and accurately isolate apathy as a distinct construct, in particular longitudinal trajectories for depression and apathy may differ.⁷³⁰ In addition to the neurobiological underpinnings, a reduction in motivation, effort and engagement can impact neuropsychological test scores.⁷³⁷ This is particularly difficult to circumvent when it has been shown that the cost of exerting mental effort in PwHD is often perceived to be high.^{439,440} Symptoms of apathy may therefore confound test scores and the reliability of cognitive estimates beyond shared neuroanatomical pathways. This chapter presents the findings of two studies which look at multidimensional apathy in pwALS and HD, and the relationship to psychiatric, behavioural and cognitive symptoms aiming to support clinical diagnostic criteria, prognostication, clinical trial evaluation and promote tool standardisation.

9.1.2 Chapter aims

This chapter addresses **Aims 3** and **6** and the corresponding **Hypotheses: 3.1-3.4** and **6.2**.

The distinct clinical and cognitive correlates of apathy in ALS and HD are explored in this transdiagnostic study. Differences in apathy profiles are expected between diseases, with greater executive apathy in HD. The chapter also examines the relationship between apathy, depression and functional ability in PwHD, and motor function in pwALS. Furthermore, it explores how apathy and behavioural disturbances relate to cognitive performance. I aim to replicate the findings of Radakovic et al. (2017)⁷³³ who found a relationship between DAS Initiation Apathy and ECAS verbal fluency scores in pwALS. Finally, it is hypothesised that domain-specific and multidimensional apathy scores will be associated with cognitive outcomes across both diseases, contributing to a better understanding of the neuropsychological overlap between motivational and behavioural symptoms, and cognitive functioning in neurodegenerative conditions.

9.2 Methods

9.2.1 Study Design

This chapter incorporates two studies with overlapping aims: pwALS in the MND Clinic and the HD-Cognition study. Study design and procedures have been outlined previously but are summarised here. Both studies have prospective, observational, quantitative designs, and HD-Cognition has additional case-control and longitudinal elements.

9.2.2 Materials

The Dimensional Apathy Scale (DAS) was used both studies as described in the methods chapter. Cut-off's for abnormality on the DAS are based on published control data. The Beaumont Behavioural Inventory (BBI) is a proxy-rated assessment of general behavioural change. Depression and fatigue were assessed in the HD study using the PHQ-9 and MFSI-SF respectively. EQ-5D-3L question 6 asked participants to rate their health state on a 0-100 scale as a measure of health-related quality of life (HR-QoL).

The ALSFRS-R and UHDRS Functional scales were used to measure functional ability in pwALS and PwHD respectively.

9.2.3 Participants

Chapter 3 provides complete information regarding recruitment, procedures and ethical considerations for each of the studies discussed here.

9.2.3.1 Study 1: pwALS in clinic

A clinic-based incident sample of pwALS who had completed an ECAS and behavioural assessment as part of their routine clinical care in the National MND Service in Beaumont Hospital between February 2023 and November 2024 were included in this study. All cases had a confirmed diagnosis of ALS by EEC and met inclusion/exclusion criteria. Of the 215 eligible pwALS available 172 cases have complete DAS-carer and ECAS data. For sample characteristics, see **Table 43** and **0**.

As the study was performed at baseline and first clinical visit, researchers were blinded to genetic status, as *C9orf72* gene status was not known for any pwALS at the time they were participating. ECAS scores falling below age- and education-based normative data cut-offs were considered 'Abnormal'.¹⁶³ Strong criteria were applied using the ECAS,

BBI and DAS; ALSci if abnormal ECAS Total or ECAS ALS-Specific domain scores⁵², ALSbi if score ≥ 7 on the BBI or ≥ 40 DAS total score, and ALSbci if they met both criteria (**Table 43**).¹⁶ Cases without complete ECAS and behavioural data were not categorised.

9.2.3.2 Study 2: HD-Cognition participants

Procedures for recruitment and inclusion/exclusion criteria for the HD-Cognition study are provided in the methods chapter. The sample in this study included manHD (n=16), preHD (n=28) and control (n=33) participants who had completed the self-rated DAS alongside an extended neuropsychological assessment. n=24 PwHD and n=20 controls also had self-rated DAS data available from consecutive timepoints, analysed in the longitudinal aspect. Full sample details shown in **Appendix 21**.

9.2.3.2.1 Genetic testing

Genotyping procedures are outlined in the methods section. Data from those who underwent clinical genetic testing for *C9orf72* in pwALS and *HTT* in PwHD are included here for sub-analyses.

9.2.4 Statistics

All cognitive tests in the extended neuropsychological test battery were z-scored using education- and/or age-stratified normative data. Age and education were included in ECAS models to control for their effects. Statistical significance was set at $p < .05$, with adjustment for multiple comparisons using the Benjamini-Hochberg False Discovery Rate (FDR) correction, where applicable.

9.2.4.1 Group comparisons

For between group comparisons, paired t-tests, Welch's t-tests and ANOVAs were used, or non-parametric equivalents (Wilcoxon rank-sum test, Kruskal-Wallis test). For categorical variables, Chi-Square tests for interdependence or Fisher's exact test were used to investigate rates of impairment between groups and Cohen's Kappa for inter-rater classification agreement.

9.2.4.2 Regression-based analysis

Linear regression assessed the extent to which cognitive performance was associated with apathy domain scores. Hierarchical models determined the extent to which effects were present above that of covariates (i.e.: PHQ-9 depression scores). Spearman's rank correlation was used for associations between non-normally distributed

variables. Test selection for the corresponding cognitive and apathy domains were based on Stuss’s model of the executive functions³³² and Radakovic et al.⁷³³ ECAS domains associated with each construct were used in the pwALS study to correspond to Radakovic et al.⁷³³ for replication. **Table 42** details these pairings. The influence of premorbid IQ (TOPF-FSIQ), age, genetic status and depressive symptoms (PHQ-9) on apathy and cognitive scores were also examined in HD, with subgroup analyses also assessing the effects of fatigue & functional ability (UHDRS-TFC) in PwHD.

Table 42: DAS Apathy domains and corresponding extended neuropsychological test/composite measures and ECAS domains.

DAS Apathy Domain	Cognitive Composite / Tests ^a		ECAS Domain ^b
Total	General	Symbol Digit Modalities (SDMT) WASI FSIQ	Total
Executive	Executive	Digit Span Backwards (WASI-IV) CWIT Inhibition errors	Executive
Emotional	Social Cognition	Reading the Mind in the Eyes (RMET) ACS-SP Affect Naming	Social Cognition
Initiation	-	Phonemic Verbal Fluency (F-A-S)	Fluency

^a Cognitive tests carried out as part of HD-Cognition study. ^b ECAS domains used in ALS study.

9.2.4.2.1 Statistical assumptions

As categorical and continuous variables were used together in some models, the assumption of homogeneity of the regression slopes was checked in each case, as well as the assumptions of linearity, and normality and homogeneity of variance of the residuals. All models were assessed for high leverage cases by evaluating Cook’s distance plots. If violations were detected, log transformations of the data were performed. Non-parametric alternatives were used if transformation did not correct the violations. Heteroscedasticity-consistent robust standard errors (HC3 SEs) are applied and reported, if necessary to address minor violations.

9.2.4.3 Longitudinal analysis: LMMs

For longitudinal analyses, to assess changes in apathy over time, linear mixed-effects models were fitted using the ‘lme4’ package in R⁶⁰⁴, with timepoint (T1, T2), group, and their interaction (group×timepoint) as fixed effects, and random intercepts to account for within-subject correlations (**Equation 13**). Additional covariates (e.g., age, PHQ-9, UHDRS, CAP-100) were included as appropriate. Subjects with complete data (DAS) at two consecutive timepoints were included in the analysis. The final sample included 44

subjects (n=88 observations) in two groups (HD (manifest at baseline n=6) n=24; Controls: n=20). Simulation studies indicate 44 subjects would yield acceptable estimates of the fixed effects.⁷³⁸ Although sufficient, it is a modest number of level-2 units, and random effects should be interpreted with some caution.⁶⁰³ Models are fit according to methods outlined in **Chapter 4**. In all cases, violation of assumptions and actions taken are reported as required.

Equation 13: *LMM for apathy over time:*

$$\text{lmer}(\text{'Apathy domain'} \sim \text{group} \times \text{timepoint} + \text{covariates} + (1 | \text{id}))$$

where, group is controls vs PwHD, and (1|id) is the random effects structure, allowing each subjects intercept to vary.

9.3 Results

9.3.1 pwALS: apathy

The distribution of pwALS carer-rated apathy scores are shown in **Figure 64**. A linear regression with DAS Total apathy as the dependent variable was constructed to assess the relationship between apathy and demographic variables:

$$\text{'DAS Total apathy domain score'} \sim \text{'age'} + \text{'education'} + \text{'sex'}$$

Overall the model explained 4.83% of the variance in apathy scores, but did not reach significance ($R^2=0.048$, $p=.061$). Age and education were not significant predictors of Total apathy (Age: $B(SE)=0.06(0.08)$, $p=.43$; Education: $B(SE)=-0.31(0.25)$, $p=.22$). Sex was significant in the model, with females being rated as less apathetic than males by 4.2 points on average, holding age and education constant ($B(SE)=-4.17(1.7)$, $p=.016$). A Welch's t-test with sex as the group shows that this was however a relatively small effect ($p=.03$, $d=0.364$). As sex was a significant predictor of DAS Total apathy, it was included in subsequent regression models as a covariate.

9.3.1.1 pwALS: Apathy abnormality rates

The overall rate of clinically significant apathy in this incident clinic-based pwALS sample ($n=159$) was 18.4% (based on carer-rated DAS). 33% of pwALS were deemed to have abnormal levels of Initiation apathy, compared to only 5.4% with abnormal Executive and Emotional apathy DAS subdomains. 36.8% of pwALS were rated as having abnormally high levels of apathy on at least one domain (**Figure 65 & Table 45**).

9.3.1.2 pwALS: Other behavioural outcomes

The Beaumont Behavioural Inventory (BBI) was completed by an informant (usually a spouse) for each pwALS whilst they were completing the ECAS ($n=198$). Scores ranged from 0 to 61 with a median score of 7. 39.4% of pwALS were deemed to have mild behavioural change (≥ 7) since the onset of symptoms of ALS, with scores for a further 15.5% indicated 'severe' behavioural change (≥ 23 ; **Figure 66**). As BBI scores were not normally distributed, Spearman's rank correlation was used to assess the relationship between apathy and behavioural symptoms generally. It showed a strong positive statistically significant association ($\rho=0.469$, $p<.001$) so that higher apathy scores were associated with higher BBI scores (**Figure 67**).

Table 43: Study 1: ALS patient sample characteristics. Whole sample at baseline visit.

n	215
Age	64.86 (10.6)
Sex (% Female)	41.9%
Years Education	14.35 (3.5)
Familial ALS (by family history; %) ^a	7.9%
C9orf72 Positive (%) ^b	19.1%
Strong Criteria Categorisation ^c	
ALSn	69 (34.6%)
ALSci	20 (10.1%)
ALSbi	81 (40.7%)
ALScbi	29 (14.6%)
NA ^e	16
ALSFRS-R ^d	38.67 (5.9)

^a n=24 missing data. ^b n=121 missing data. ^c Strong Criteria Categorisations were made based on the ECAS, BBI and DAS, they do not reflect full categorisation according to Strong Criteria (2017); ^d n=62 missing data. ^e NA for cases without complete ECAS and behavioural data and not ALSc/bi; ALSn=ALS without cognitive or behavioural impairment. ALSFRS-R = ALS-Functional Rating Scale-revised. Data presented as M(SD) unless otherwise stated. Extended patient characteristics in **Appendix 22 & 23**.

Table 44: Results table for pwALS with complete DAS apathy and ECAS scores at baseline clinic visit.

	M(SD) / %	% Abnormal
DAS domain (carer-rated; n)	159	-
Total	28.9 (10.5)	18.4%
Executive	5.62 (4.2)	5.4%
Emotional	9.72 (3.78)	5.4%
Initiation	14.0 (5.0)	33%
BBI (n)	169	-
BBI post	11.87 (12.0)	-
Normal		44.4%
Mild		40.4%
Severe		15.2%
ECAS baseline T1 (Version A; n)	171	-
Mode response (% verbal)	89.6%	-
ECAS Total	102.84 (16.44)	22.8%
ALS-Specific	77.3 (13.18)	18.7%
Language	25.18 (3.6)	18.7%
Fluency	16.03 (5.0)	24%
Executive	36.0 (7.1)	6.4%
Social Cognition	10.8 (2.32)	-
ALS-non-Specific	25.66 (5.3)	22.8%
Memory	14.18 (5.0)	19.9%
Visuospatial	11.49 (0.94)	4.7%
Complete DAS & BBI T1 (n)	158	-
Complete ECAS, DAS, BBI T1 (n)	152	-

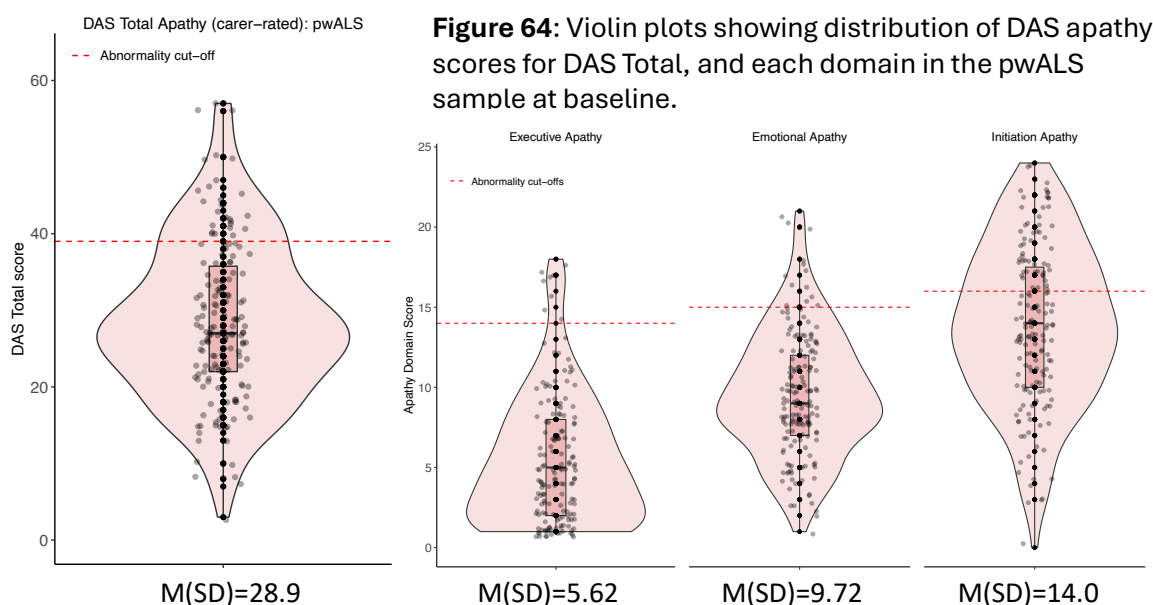
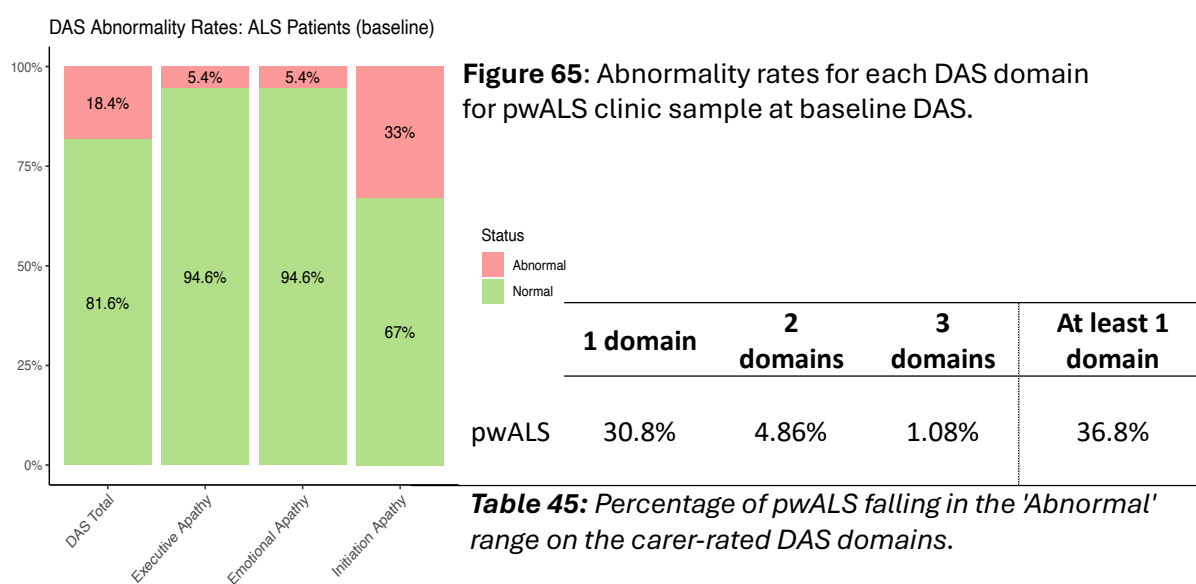


Figure 64: Violin plots showing distribution of DAS apathy scores for DAS Total, and each domain in the pwALS sample at baseline.



9.3.2 pwALS: Cognitive outcomes

n=171 pwALS had ECAS A baseline data available at their first visit to clinic. Rates of impairment were calculated based on published normative data for age and years of education.¹⁶³ **Figure 69** shows 22.8% of pwALS performed in the Abnormal range at their first visit to the clinic, with 18.7% impaired on the ALS-specific subtest and 22.8% on the ALS-non-specific subtest.

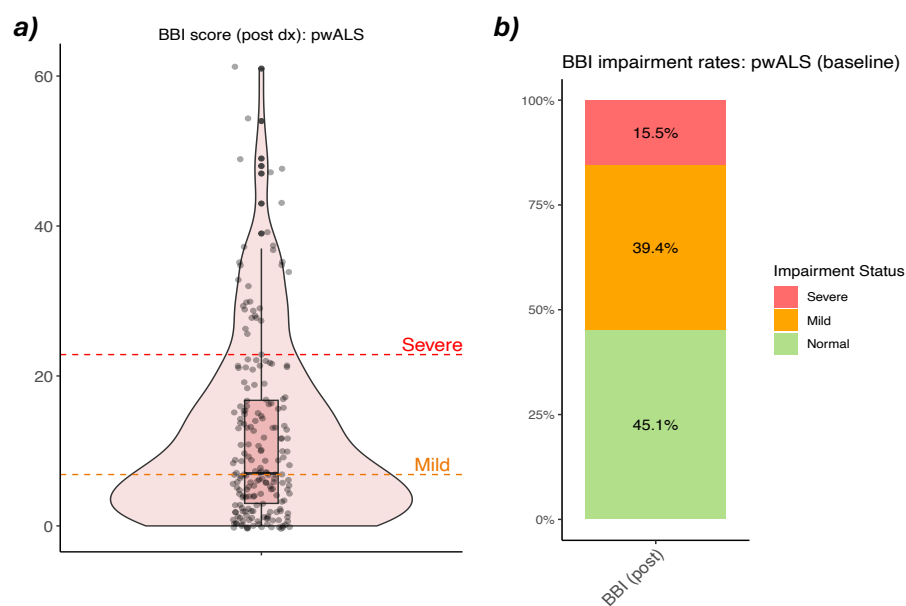


Figure 66: BBI behavioural score plots.

a) violin plot showing score distribution in pwALS; **b)** % of pwALS with scores falling into each impairment category.

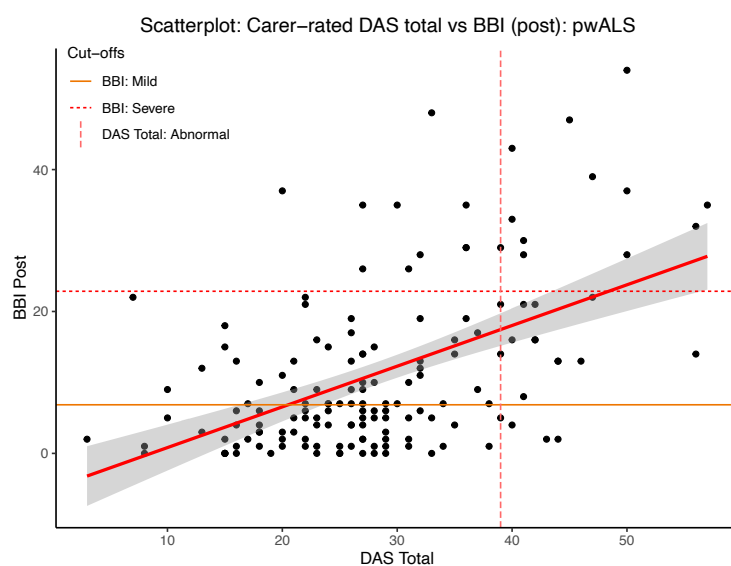


Figure 67: Scatterplot DAS Total apathy & BBI scores for pwALS.

Both carer-rated measures. Cut-off lines based on published cut-offs above which signifies impairment.

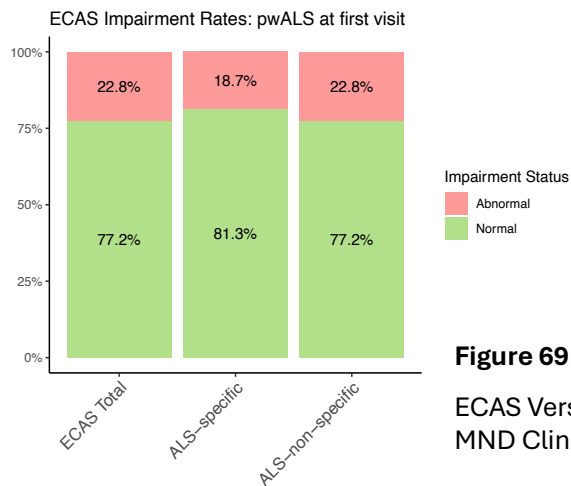


Figure 69: Impairment rates on ECAS.

ECAS Version A at pwALS first visit to the MND Clinic.

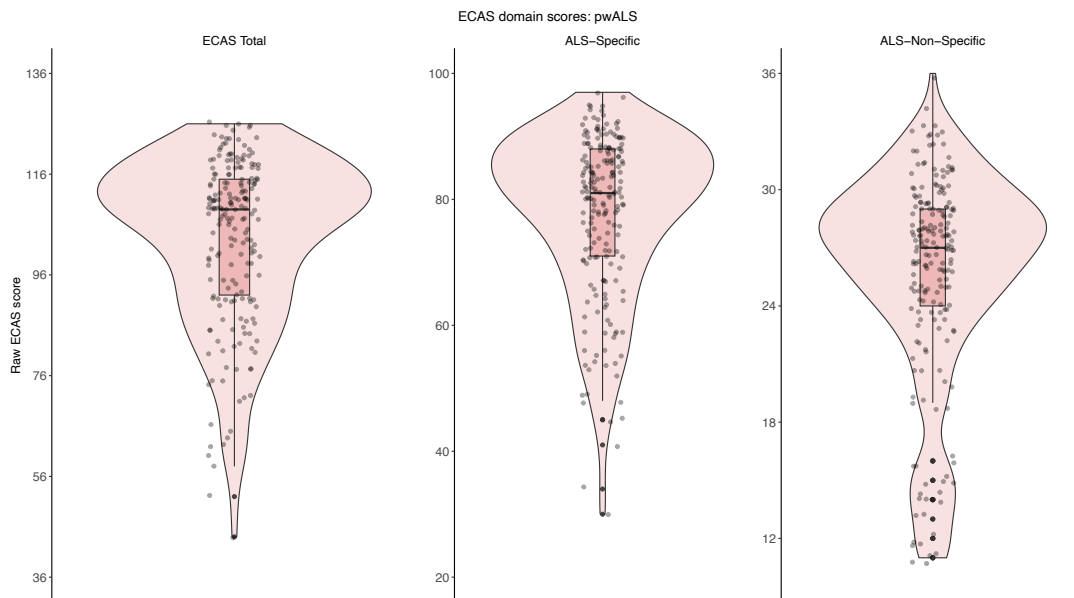


Figure 68: Violin plots of ECAS domain scores for pwALS.

9.3.3 pwALS: Cognitive function & apathy

Simple linear regressions were constructed to assess the extent that cognitive test scores, in this case, the ECAS, are related to DAS apathy domain scores. n=152 pwALS had complete ECAS and DAS data at their baseline visit to the clinic. Age and education are not associated with DAS Total apathy scores, so although these variables are significant contributors to ECAS scores, they were not included as covariates. Sex was included as a covariate in the models for its' weak relation to cognitive scores, and also symptoms of apathy in this sample:

$$\text{'Apathy domain score'} \sim \text{'ECAS domain score'} + \text{'sex'}$$

For DAS Total apathy, robust standard errors were applied to account for non-constant variance of the residuals (heteroscedasticity). The model significantly predicted DAS Total apathy scores, accounting for 13.4% of the variance (robust SEs applied: $R^2=0.134$, $p_{HC3}<.001$). ECAS Total score was a significant predictor, with DAS Total scores decreasing by 0.21 points for every unit increase in ECAS Total score ($B(SE)=-0.21(0.05)$, $p_{HC3}<.001$; **Table 46**).

Table 46: Regression model for ECAS Total score and sex as predictors for DAS Total apathy. Robust HC3 standard errors applied.

	R^2	$B(SE)$	$t(149)$	β	p_{HC3}
Total Apathy model	0.134				<.001
Constant		51.42(5.21)	9.86	0.16	<.001
ECAS Total		-0.21(0.05)	-4.2	-0.32	<.001
Sex (female)		-3.84(1.59)	-2.41	-0.37	.015

Next, linear regression models were created for each corresponding pair of apathy and ECAS domains (**Table 42**), again holding sex constant as a covariate. All assumptions of linear regression held for each model, however, the residuals were not normally distributed in the Executive model. Given the sample size is large ($n=152$), normality can be assumed by the Central Limit Theorem. Additionally, regression is robust to such minor deviations, and so it was retained.

There were small but significant effects for two of the three models after correction for multiple comparisons (Executive and Emotional/Social Cognitive; **Table 47**). The Initiation apathy model approached significance ($R^2=0.035$, $p_{adj}=.072$), as did ECAS Fluency in predicting Initiation apathy scores ($B(SE)=-0.16(0.08)$, $p_{adj}=.077$). Females had significantly lower Emotional apathy in the social cognitive model ($p_{adj}=.024$) and Total apathy in the total model ($p_{adj}=.017$). A small but significant association was evident for ECAS executive scores and Executive apathy on the DAS ($\beta=-0.21$, $p_{adj}=.024$). Higher ECAS executive scores were associated with lower DAS Executive apathy, which decreased by 0.12 for each point increase on the ECAS executive domain.

Table 47: Multiple regression models testing the association of ECAS cognitive domains and their respective DAS subdomain, holding sex constant.

ECAS Cognitive domain	R ²	B(SE)	t(149)	β	p ^a
Executive apathy model	0.058				.011
Constant		10.1(1.69)	5.98	.11	<.001
ECAS: Executive domain		-0.12(0.05)	-2.61	-0.21	.01
Sex (female)		-1.03(0.65)	-1.58	-0.25	.12
Emotional apathy model	0.042				.041
Constant		10.00(1.52)	6.57	0.18	<.001
ECAS: Social Cognition domain		0.03(0.14)	0.21	0.02	.83
Sex (female)		-1.60(0.63)	-2.54	-0.41	.012
Initiation apathy model	0.035				.072
Constant		16.78(1.40)	11.99	0.09	<.001
ECAS: Fluency domain		-0.16(0.08)	-1.96	-0.16	.051
Sex (female)		-0.95(0.80)	-1.19	-0.19	.24

^a p-values before correction for the FDR.

9.3.3.1 *pwALS: Cognitive, behavioural & physical function*

Lastly, to alter the direction, examining the factors affecting cognitive test scores, and if Total apathy was among them, a multiple regression model was created. Onset site was coded as ‘Bulbar’, ‘Spinal’ and ‘Other’ to reduce distortion of regression estimates. LRT ANOVA model comparisons found neither sex nor general behavioural impairment (BBI scores) significantly improved the models, nor had independent effects on ECAS scores. The final model was as so:

$$\text{‘ECAS total’} \sim \text{‘Age’} + \text{‘Years education’} + \text{‘Site of Onset’} + \text{‘DAS Total score’}$$

High-leverage residuals were present, and so a sensitivity analysis was performed by robust regression. The strength and direction of the relationships remained unchanged, and so the standard model is reported here. The model was highly significant, and explained 25.6% of the variance in ECAS Scores ($R^2=0.256$, $p<.001$). After onset site, DAS Total apathy ($\beta = -0.30$, $p<.001$) had the largest significant independent effect on ECAS Total score, followed by years of education and age (both $p<.03$; **Table 48**), with ECAS scores decreasing 0.46 points per unit increase in DAS Total apathy score and improving 1.24 points per additional year of education. Bulbar onset pwALS scored between 6.2 and 6.9 points less on the ECAS when controlling for all other factors.

Table 48: ECAS Total and DAS Total apathy linear regression model

	R ²	B(SE)	β	p
Full model	0.256			<.001
Constant		108.69(9.93)	-0.29	<.001
Age		-0.23(0.11)	-0.16	.03
Education		1.24(0.35)	0.26	<.001
Onset site: Spinal		6.17(2.69)	0.38	.023
Onset site: Other		6.86(3.76)	0.43	.069
DAS Total Apathy		-0.46(0.11)	-0.30	<.001

NOTE: The base model was for pwALS with Bulbar onset. n=152 pwALS at baseline clinic visit.

A hierarchical regression sub-analysis was performed for pwALS with available ALSFRS-R data (n=125) to control for the effects of disease progression, adding ALSFRS-R in the last step (**Table 49**):

‘ECAS total’ ~ ‘Age’ + ‘Years education’ + ‘Site of Onset’ + ‘DAS Total score’ + ‘ALSFRS-R’

The base model (age and education) was highly significant ($R^2=0.145$, $p<.001$). Step 1 added site of onset to the model in three categories (Bulbar, Spinal, Other). Although this addition did not significantly improve model fit ($p=.074$), it added 3.8% to the explained variance overall ($R^2=0.184$, $p<.001$). As an important clinical predictor, it is retained. Step 2 added DAS Total apathy, which added an additional 9.63% explained variance, a highly significant improvement (ΔR^2 : $p<.001$; whole model: $R^2=0.28$, $p<.001$). Step 3 added ALSFRS-R scores—a measure of physical impairment. It did not contribute significantly to the model ($p=.737$), and all prior predictor relationships remained unchanged. The overall model remained highly significant ($R^2=0.281$, $p<.001$). This indicates ECAS scores are not related to functional ability when controlling for age, education, site of onset and DAS Total apathy. For every unit increase in DAS Total apathy, ECAS scores fell by nearly 0.5 points, holding all other things constant, having a large individual contribution to apathy ($\beta = -0.31$, 95%CI: [-0.48, -0.15], $p<.001$).

9.3.4 pwALS: Apathy, behaviour & functional ability

ALSFRS-R data was available for 125 of the 159 cases who had complete DAS data at first clinic visit (**Table 44**). Spearman correlations between ALSFRS-R total score and each DAS sub-domain showed weak but significant inverse univariate associations for Executive ($\rho = -0.27$, $p_{\text{adj}}=.004$) and Initiation ($\rho = -0.26$, $p_{\text{adj}}=.004$) apathy, but not

Emotional apathy ($\rho = -0.009$, $p_{\text{adj}} = .90$). BBI scores also showed a weak negative association ($\rho = -0.36$, $p_{\text{adj}} < .001$).

Table 49: Hierarchical multiple regression for DAS Apathy Total and ECAS Total score assessing the effects of motor dysfunction.

	ΔR^2	B (SE)	β	p
Step 1	0.038			.074
Constant		117.7(11.24)	-0.32	<.001
Age		-0.29(0.12)	-0.18	.040
Education		1.16(0.41)	0.25	.004
Onset site: Spinal		6.71(3.03)	0.43	.029
Onset site: Other		8.05(5.45)	0.52	.143
Step 2	0.096			<.001
Constant		111.11(11.27)	-0.33	<.001
Age		-0.24(0.11)	-0.17	.039
Education		1.09(0.4)	0.23	.007
Onset site: Spinal		6.82(2.86)	0.44	.019
Onset site: Other		10.79(5.19)	0.69	.040
DAS Total Apathy		-0.47(0.12)	-0.32	<.001
Step 3	0.0007			.737
Constant		107.33(15.93)	-0.34	<.001
Age		-0.24(0.12)	-0.17	.044
Education		1.1(0.4)	0.23	.007
Onset site: Spinal		11.26(5.39)	0.45	.018
Onset site: Other		6.96(2.9)	0.72	.039
DAS Total Apathy		-0.47(0.12)	-0.31	<.001
ALSFRS-R		0.08(0.23)	0.03	.737

NOTE: The intercept at each step was for Bulbar onset pwALS. $n = 119$ pwALS at baseline who had complete ECAS, DAS and ALSFRS-R data.

Multiple regression was carried out with ALSFRS-R as the DV and each of the three DAS domains and BBI score as predictors. All assumptions were met, with the exception of normality of the residuals, which deviated slightly from normal. Neither log transformation nor robust regression ameliorated this. However, linear regression is robust to mild non-normality in large samples such as this. Overall the model was significant, explaining 12.3% of the variance in ALSFRS-R scores ($R^2 = 0.123$, $p = .003$). Initiation apathy and BBI scores were the only significant individual predictors of ALSFRS-R, with equal effect ($\beta = -0.26$; **Table 50**).

Table 50: Multiple regression model for ALSFRS-R scores with DAS subdomains as predictors.

	R ²	B(SE)	t(149)	β	p
ALSFRS-R model	.123				.003
Constant		42.33(1.54)	27.52	.	<.001
DAS Executive		0.02(0.18)	0.14	0.02	.892
DAS Emotional		0.19(0.16)	1.17	0.12	.245
DAS Initiation		-0.29(0.12)	-2.44	-0.26	.016
BBI (carer-rated)		-0.14(0.06)	-2.29	-0.26	.024

NOTE: BBI and Executive apathy are highly related. Although there was no multicollinearity, BBI scores reduce the independent variance contributed by Executive apathy in the model.

9.3.4.1 pwALS: Abnormality classifications for cognition, apathy & behaviour

Chi-square tests for independence assessed DAS Total apathy and BBI abnormality classifications with relation to ECAS Abnormality (see **Table 51**). DAS Total and ECAS classifications were related, and not significantly independent ($\chi^2(152)=6.08$, $p=.0137$), suggesting participants with cognitive impairment were more likely to also display clinically significant apathy. BBI scores on the other hand were independent, as the null hypothesis was not rejected ($\chi^2(152)=2.84$, $p=.242$), indicating general behavioural impairment occurs with or without cognitive impairment, as measured by the ECAS.

Table 51: Abnormality classifications for DAS Apathy and BBI as they relate to the ECAS abnormality classifications. n (% of test total).

ECAS	DAS Total		BBI Status		
	Normal	Abnormal	Normal	Mild	Severe
Normal	103 (67.8%)	14 (9.2%)	59 (38.8%)	42 (27.6%)	16 (10.5%)
Abnormal	24 (15.8%)	11 (7.2%)	12 (7.9%)	17 (11.2%)	6 (3.9%)

9.3.4.2 pwALS: The effect of C9orf72

n=71 pwALS had genotype data available, 46.7% of the total sample. 16.9% (n=12) tested positive for the C9orf72HRE. The two groups based on genetic status (C9+/C9-) were compared at their baseline assessment. The C9+ group trended towards being older, had a higher onset age, and were more likely to have bulbar-onset ALS, but these did not reach significance (all $p>.05$; **Appendix 23**). There were no other demographic or clinical

differences between the genotypes. The groups differed only on the visuospatial domain of the ECAS, with C9+ (Md=11) scoring lower than C9- pwALS (Md=12; W=176, $p_{\text{adj}}=.01$). There were no significant differences between the groups on the BBI, DAS or other ECAS domains (all $p>.05$).

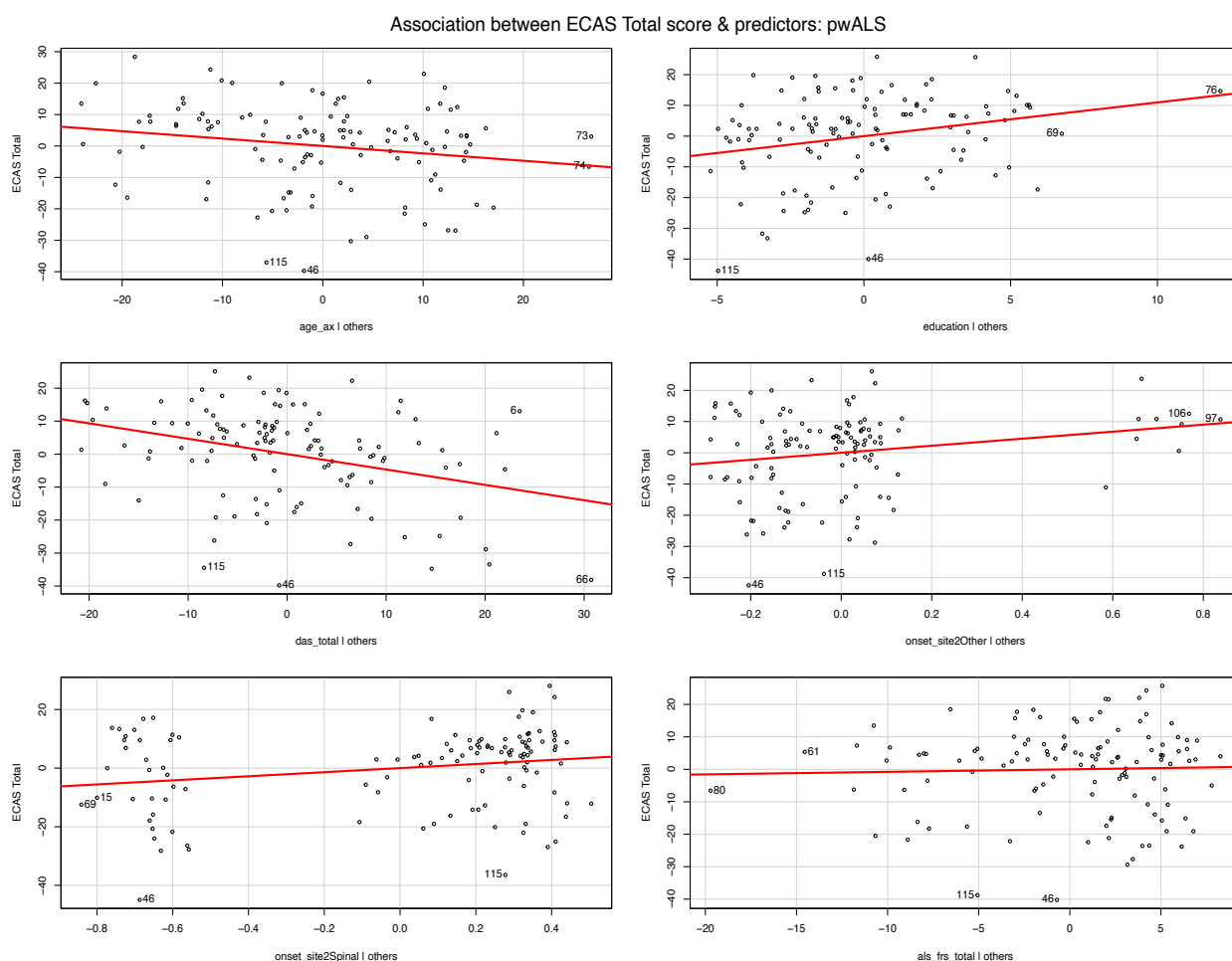


Figure 70: Added variable plot for final pwALS ECAS regression model:

NOTE: ‘ECAS total’ ~ ‘Age’ + ‘Years education’ + ‘DAS Total score’ + ‘Site of Onset’ + ‘ALSFRS-R’. The plot shows the independent effects of each predictor on ECAS Total score by holding every other predictor constant.

9.3.5 PwHD: Apathy

In the HD-Cognition study, control, preHD and manHD participants completed the self-reported version of the DAS as part of the extended neuropsychological test battery. Scores for the control group were consistent with published normative scores and clinical cut-offs, with only one control scoring in the Abnormal range on one domain (Initiation

apathy).^{154,441} This supports the reliability and validity of the use of clinical cut-off scores in this study.

Groups were matched for age, sex and education (though manHD participants trended towards having lower education compared to controls (p.adj=.057). Controls had significantly higher TOPF scores at T1 ($p < .001$, $\omega^2 = 0.19$) compared to both manifest (p.adj<.001) and premanifest (p.adj=.002) groups. Therefore it was used as a covariate in all analyses with cognitive variables. Welch's t-test with sex as group was significant with a moderate effect size ($p = .001$, $d = 0.798$). Females were rated as having 5.21 points lower DAS Total scores relative to males, accounting for the effects of group, with moderate effect size (EMM estimate=5.21(2.19), $p = .02$, $d = 0.6$). As groups were matched on sex, this was not controlled for in group comparisons. Linear regressions were constructed which controlled for premorbid IQ estimates (TOPF-FSIQ), isolating the effect group on apathy scores as follows:

$$\text{'apathy domain score'} \sim \text{'group'} + \text{'premorbid IQ estimate'}$$

Results show TOPF-FSIQ did not significantly predict DAS scores in any domain, and so group differences in TOPF-FSIQ are unlikely to confound comparisons here. The overall models were significant for Total ($R^2 = 0.25$, $p < .001$), Executive ($R^2 = 0.26$, $p < .001$) and Initiation ($R^2 = 0.15$, $p = .01$) apathy domains, but not Emotional apathy ($R^2 = 0.056$, $p = .25$), although, manifest participants did endorse higher levels of emotional apathy than controls ($B = 2.67$, 95%CI:[0.03,5.31], $p = .047$). Post-hoc comparisons with Benjamini-Hochberg correction for FDR showed manHD participants differed from the control and preHD groups on all other domains (Total, Executive & Initiation; all p.adj<.01). Premanifest and control participants differed significantly on the Executive (p.adj=.01) and Total (p.adj=.027) domains, but not the Initiation Apathy domain (p.adj=.17) which approached significance after correction for multiple comparison (**Table 54 & Figure 72**).

9.3.5.1 PwHD: Apathy abnormality rates

62.5% of the manifest participants and 26.9% of the premanifest participants rated themselves as having clinically significant apathy in at least one of the three DAS subdomains (**Figure 71, Table 52**). Only 1 control participant fell in the abnormal range on one DAS domain (Initiation apathy). Fisher's exact test found significant group differences in abnormality rates for each domain, but with slightly smaller effect for Emotional apathy ($V = 0.27$, p.adj=.04). The strength of the associations for Executive, Initiation and Total apathy were larger and moderate (Cramer's V: 0.38–0.45; all p.adj<.003; **Table 54**).

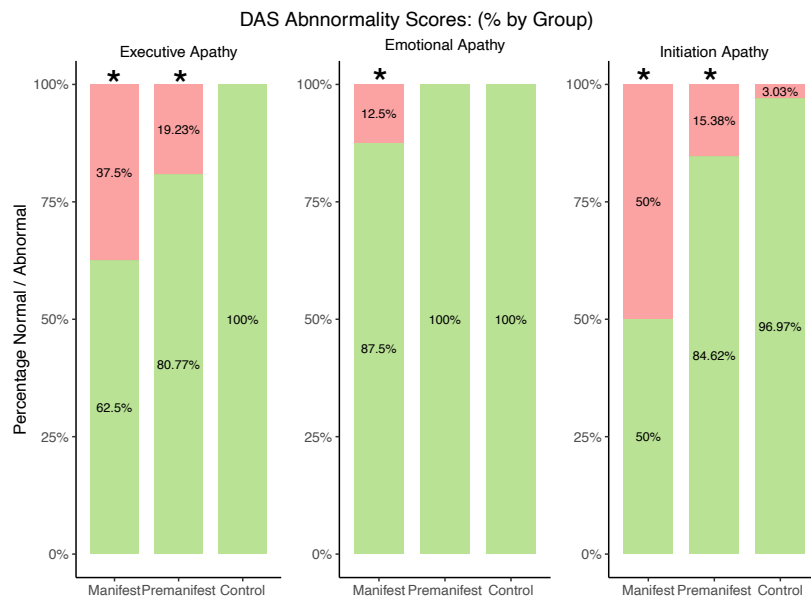


Figure 71: Rates of impairment on DAS apathy in HD-Cognition study. *: $p < .05$ vs controls

Table 52: Percentage of cases in HD cohort falling in the 'Abnormal' range on the DAS by group.

Group	Any 1 domain	Any 2 domains	Any 3 domains	At least 1 domain
Manifest	37.5	12.5	12.5	62.5
Premanifest	19.2	7.69	0	26.9
Control	3.03	0	0	3.03

9.3.5.2 PwHD: Other outcomes: depression & anxiety

PHQ-9 scores were not normally distributed, and clustered at the lower end of the scale. A Kruskal-Wallis test found a significant group difference ($\chi^2_{(2)}=12$, $p=.003$). Post-hoc Wilcoxon rank sum test with continuity correction showed control ($p_{\text{adj}}=.002$) and premanifest ($p_{\text{adj}}=.026$) groups different significantly from the manifest group, but not each other ($p_{\text{adj}}=.265$; **Table 54**). Published abnormality cut-offs were applied, finding PwHD had higher rates of depressive symptoms than controls by Fisher's exact test with Monte Carlo simulation ($p=.008$, Cramer's $V=0.30$), with 56% of manHD, 23% of preHD and 6% of controls endorsing clinically significant depression. Kruskal-Wallis test found groups differed on the GAD-7 also ($p=.004$), with post-hoc pairwise comparisons showing significant differences between manifest and control groups ($p=.005$). Scores in the premanifest group fell between the manifest and control groups, with differences approaching significance ($p=.065$ and $p=.102$ respectively) and rates in each group were similar to that of depression scores (**Table 54**, **Figure 71**). There was a very strong significant correlation between GAD-7 and PHQ-9 scores ($\rho=0.77$, 95%CI:[0.66–0.85], $p<.001$), suggesting symptoms of depression and anxiety co-occurred in this sample.

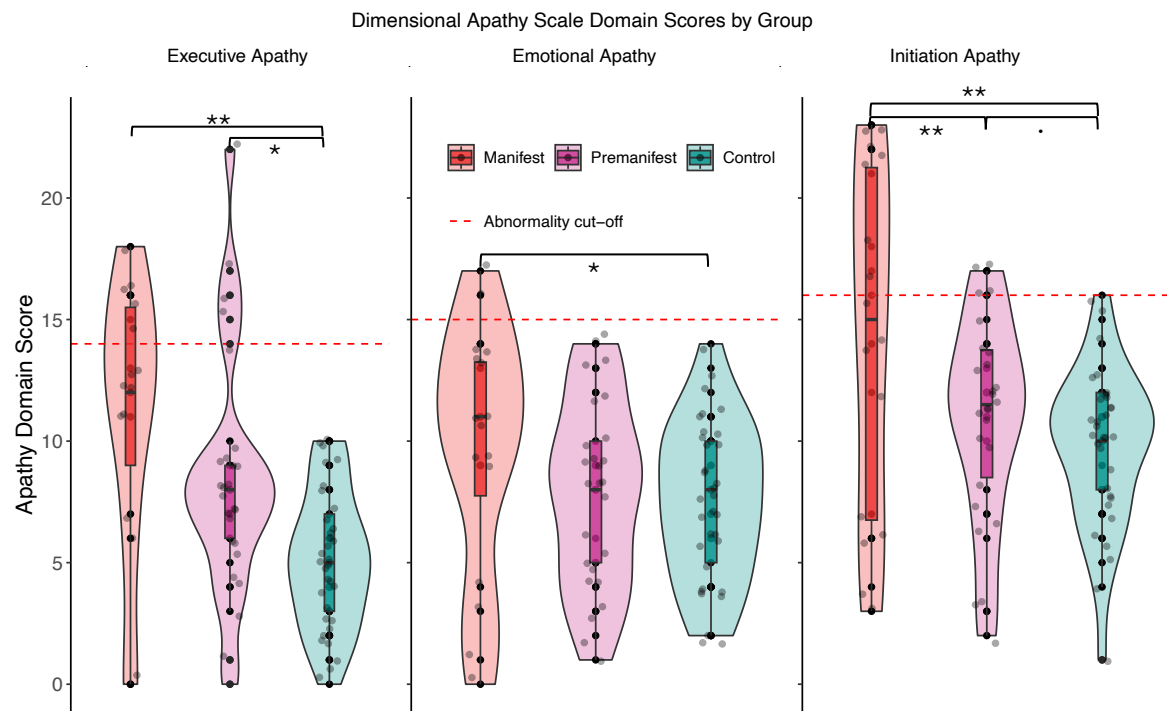


Figure 72: Baseline DAS domain scores in HD-Cognition groups, with published abnormality cut-off by group **: $p < .001$; *: $p < .05$; .: p approaches significance.

9.3.5.2.1 PwHD: Apathy & depression

Hierarchical regression (**Table 53**) found that depressive symptoms accounted for 31% of the variance in Total apathy scores alone ($p < .001$). Step 2 added in group, which remained a significant predictor when controlling for depressive scores in the manHD groups, explaining an additional 7% of the variance in apathy scores overall. This suggests group differences in apathy are not attributable only to depressive symptoms. Despite a relative drop of 4.52 points in Total apathy when holding depression scores (PHQ-9) constant, the main effect for the premanifest group did not reach significance ($B(SE) = -4.52(2.92)$, $p = .13$), and EMMs showed they no longer differed from controls ($p_{adj} = .13$). Overall, the model explained 37.4% of the variance in DAS Total scores ($adj.R^2 = 0.37$, $p < .001$; **Figure 74**).

Similar patterns were found for domains of Executive and Initiation apathy, but not for Emotional apathy, which had no significant relationship to group or PHQ-9 scores ($p > .05$). EMMs found that, even when holding PHQ-9 score constant, preHD rated higher on the Executive apathy subscale compared to controls ($B(SE) = -2.42(0.96)$, $p_{adj} = .023$), but not on the Initiation apathy subscale ($p = .58$).

Table 53: Hierarchal multiple regression for DAS Total apathy & PHQ-9 depressive symptoms. Step 2: ‘apathy’ ~ ‘depression’ + ‘group’

DAS Total Apathy	ΔR^2	B(SE)	β	p
Step 1	0.31			
Constant		21.25(1.43)	-1.23 ⁻¹⁶	< .001
PHQ-9 Total		1.01(0.18)	0.56	< .001
Step 2	0.07			
Constant		27.66(2.97)	0.48	< .001
PHQ-9 Total		0.77(0.19)	0.42	< .001
Group: Premanifest		-4.52(2.92)	-0.42	.13
Group: Control		-7.97(2.96)	-0.75	.009

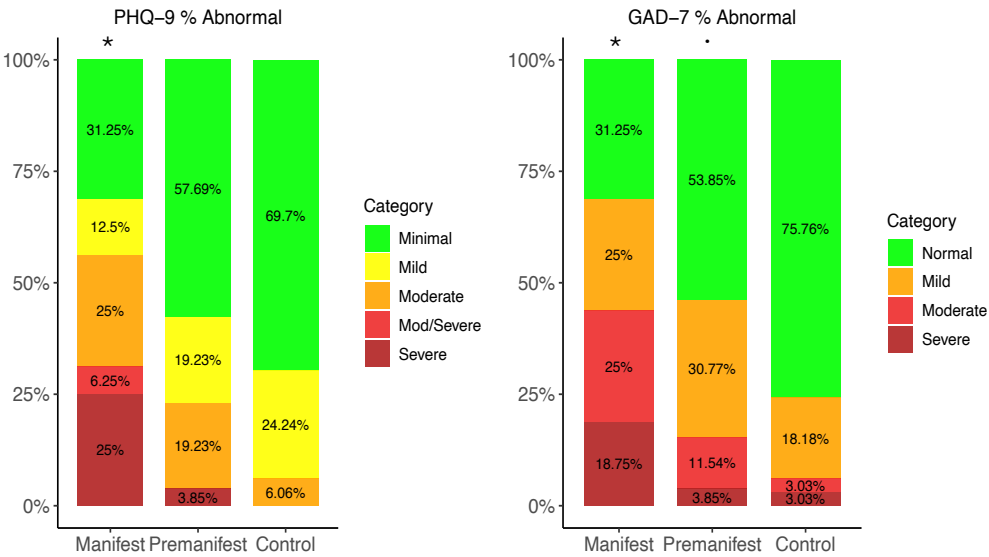


Figure 73: Rates of depression & anxiety in the HD sample. * p<.05 vs controls.

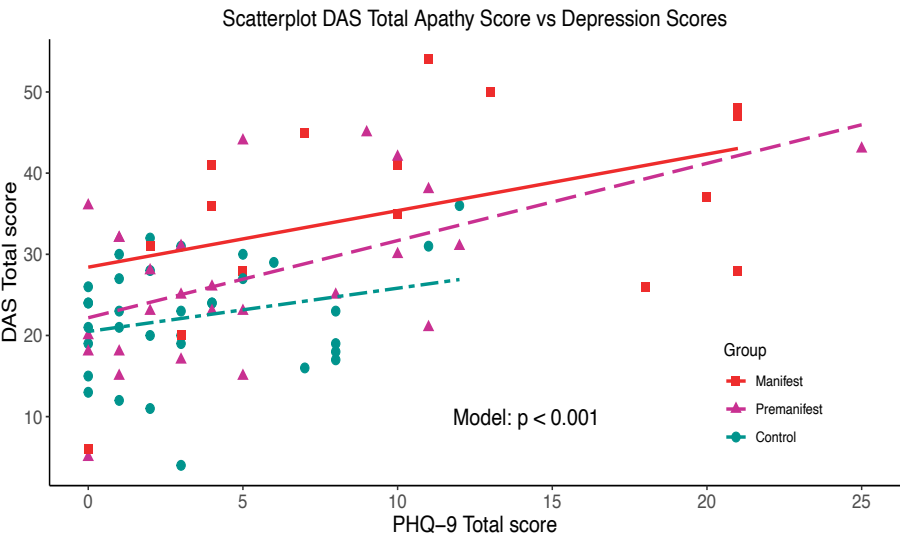


Figure 74: Scatterplot of relationship between self-rated DAS Total apathy and PHQ-9 scores for PwHD.

9.3.6 *PwHD: Cognitive outcomes*

9.3.6.1 *PwHD: Between group comparisons*

Multiple linear regressions, controlling for TOPF-FSIQ, found significant main effects for group in all cognitive tests/composites of interest (all $p_{\text{adj}} < .001$; **Table 54**). Post-hoc comparisons of estimated marginal means with Benjamini-Hochberg correction showed that this was driven by manHD (all $p_{\text{adj}} < .01$). Premanifest and control groups differed significantly only on the SDMT (EMM estimate(SE) = $-0.83(0.41)$, $p = .047$), but this did not remain significant after controlling for multiple comparisons ($p_{\text{adj}} = .072$).

9.3.6.2 *PwHD: Cognitive function & apathy*

To assess the extent to which apathy is predicted by cognitive function (controlling for premorbid IQ) in these three groups, further linear regressions were constructed with the domain-specific cognitive composites (**Table 42**). Domain model outcomes are shown in **Figure 75** and constructed as follows:

$$\text{'DAS domain score'} \sim \text{'Cognitive composite/score'} + \text{'TOPF-FSIQ'}$$

In the Executive apathy/cognitive composite model the executive composite score was significantly predictive of Executive apathy, holding premorbid IQ estimates constant ($B(\text{SE}) = -1.27(0.71)$, $p_{\text{adj}} = .036$; $\beta = -0.3$, 95%CI: $[-0.54, -0.05]$). There was a trend towards the social cognition composite score to account for a portion of the variance in Emotional apathy scores, but was not significant after correction in this model ($p_{\text{adj}} = .056$).

The model for Initiation apathy and phonemic verbal fluency (FAS scores), controlling for TOPF-FSIQ was significant overall, accounting for 9% of the variance in initiation apathy scores ($R^2 = 0.09$, $p = .031$). There was a significant main effect of FAS, which indicated that, after adjusting for TOPF, higher phonemic fluency scores were significantly associated with lower Initiation apathy scores, with a 1.29 point decrease in initiation apathy scores per unit increase in fluency scores ($\beta = -0.33$, 95%CI: $[-0.57, -0.09]$, $p_{\text{adj}} = .036$). As for the general cognitive composite score, the overall model did not explain significant variation in DAS Total apathy scores ($R^2 = .009$, $p = .74$), nor was there a main effect of the total composite score ($p_{\text{adj}} = .323$).

Table 54: Results table for the HD-Cognition study apathy analysis.

	Controls	Premanifest	Manifest	df	F/ χ^2	R ² / η^2 /V ^d	p
n	33	28	14	-	-	-	-
TOPF-FSIQ M(SD)	112.6(9.5)	103.5(12.0)	100.0(10)	2,72	9.69	-	<.001
							p.adj^b
DAS Subtest (Self; Raw Score)							
Total	22.3(6.9)	27.2(10.1) ^c	35.8(12.5)	3,71	7.85	0.25	<.001
(% abnormal)	0%	15.40%	43.80%	-	-	0.44	<.001
Executive	5.1(2.8)	8.4(5.0) ^c	11.9(6.3)	3,71	8.46	0.26	<.001
(% abnormal)	0%	19.20%	37.50%	-	-	0.38	<.001
Emotional	7.5(3.2)	7.9(3.8)	9.7(5.2)	3,71	1.41	0.06	.247
(% abnormal)	0%	0%	12.50%	-	-	0.27	.04
Initiation	9.7(3.2)	10.9(4.2)	14.3(7.2)	3,71	4.12	0.15	.01
(% abnormal)	3%	15.40%	50%	-	-	0.45	<.001
PHQ-9 (raw score; Median (IQR))	3(4)	3.5(7.75)	10(14.5)	2	12	0.14	.003
(% $\geq$ Moderate)	6.10%	23%	56.20%	-	-	0.3	.008
GAD-7 (raw score; Median (IQR))	1(4)	3.5(5.75)	7.5(9.5)	2	11	0.124	.004
(% $\geq$ Moderate)	6%	15.30%	43.80%	-	-	0.23	.02
Cognitive scores (z-scores)							
General (FSIQ-2; Standard Scores)	113.2(10.5)	104.9(14.5)	88.1(14.2) ^e	3,68	25.1	0.53	<.001
General 2 (SDMT)	-1.06(1.2)	-1.69(1.7)	-4.55(1.6)	3,71	23.1	0.49	<.001
General composite	-0.09(0.6)	-0.69(1.2)	-2.51(0.98)	3,68	21.8	0.49	<.001
Executive (Digit Span Backwards)	0.62(1.1)	-0.05(0.9)	-1.15(0.8)	3,71	13.5	0.36	<.001
Executive 2 (CWIT Inhibition Errors)	0.29(0.56)	0.34(0.56)	-1.06(1.33)	3,71	13.8	0.37	<.001
Executive composite	0.46(0.7)	0.14(0.6)	-1.1(0.9)	3,71	22.6	0.49	<.001
Emotional (RMET)	0.48(0.76)	0.26(0.9)	-1.76(1.3)	3,71	23.3	0.50	<.001
Emotional 2 (Affect Naming)	0.25(0.9)	-0.04(1.1)	-2.0(0.9)	3,66	25	0.53	<.001
Emotional composite	0.36(0.7)	0.11(0.9)	-1.96(1.0)	3,66	33.1	0.6	<.001
Initiation (Phonemic Fluency)	0.26(0.9)	-0.22(1.1)	-1.77(0.9)	3,71	17.1	0.42	<.001

NOTE: Scores presented as M(SD) of standard scores/z-scores. ^a based on normative data stratified for age and education. ^b reported p-values are from models holding TOPF-FSIQ constant and following BH correction. ^c Premanifest differ from controls. ^d R² values are the variance explained by models, controlling for TOPF with group as the independent variable and test score as the dependent variable ['test variable' ~ group + TOPF-FSIQ] and Cramer's-V as a measure of effect size for categorical comparisons. ^e n=3 Manifest participants did not have complete WASI-FSIQ data available.

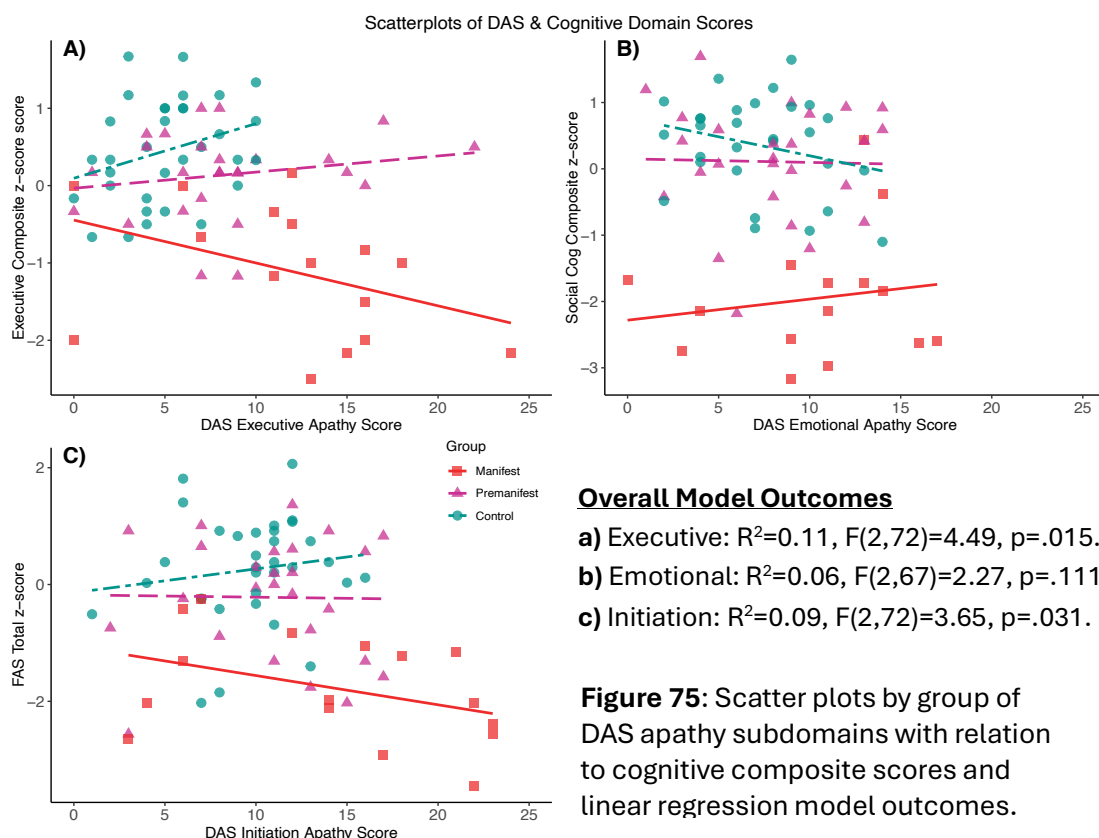
9.3.7 PwHD: Apathy, functional ability & disease progression

Subgroup analyses combined PwHD (premanifest and manifest) to assess the effects of functional scores on apathy. Simple linear regression including Total Functional Capacity (TFC) and UHDRS Functional Total Score were inversely associated with self-reported apathy (TFC: B= -1.98, p=.008; UHDRS Function: B= -1.33, p=.017) suggesting a relationship to functional ability in general. For every unit decrease in TFC, there was a 2 point increase in Total apathy scores, explaining 16.4% of the variance.

Neither functional measure (TFC nor UHDRS Function) was significant in a multiple regression which included group (HD vs controls) and depression score (TFC: B=

-1.0, $p=.113$), with the model as a whole explaining 35% of the variance in DAS Total scores. As above, depression accounted for a large portion of the variance in apathy domains, and negated the effect of TFC or UHDRS Function. Although the control group scored 3.97 points less on the DAS, this only approached significance in this model ($B(SE)=-3.97(2.17)$, $p=.072$).

The UHDRS function scales are relatively insensitive to the premanifest phase however. To explore this effect more sensitively across disease stages, estimates of CAG-associated disease progression (CAP100)³¹⁵ and Langbehn et al.'s.^{71,314} parametric survival model of age at predicted onset were assessed in separate simple linear regression models. Neither predictor could explain significant portions of the variance in PwHD ($n=34$) baseline Total Apathy scores ($p>.91$). Together these findings suggest that depression and apathy scores are highly associated, but relatively independent of disease-related factors.



9.3.8 PwHD: Apathy, fatigue & HR-QoL

High levels of fatigue (MFSI-SF) were endorsed by manHD participants ($p_{adj}=.022$). Fatigue was elevated in the premanifest group, but not significantly so ($p_{adj}=.08$) and was strongly related to DAS Total apathy overall ($p_{adj}=.034$). The

Executive apathy subdomain of the DAS drove this relationship as the strongest predictor ($p_{\text{adj}} < .001$). The 'Mental' fatigue and 'Vigour' subdomains of the MFSI-SF were positively and negatively associated respectively with DAS Total apathy and SDMT total scores in multiple regression models ($p_{\text{adj}} < .05$). Whereas 'Mental' and 'Emotional' fatigue were associated with UHDRS Function scores ($p_{\text{adj}} < .05$).

Examining cognitive variables, the same process for linear regression as above was applied. Differences remained for all groups when holding apathy subdomains & fatigue constant for speed of processing ($p_{\text{adj}} < .001$), and for manifest group only for executive function ($p_{\text{adj}} < .001$), social cognition ($p_{\text{adj}} < .001$) and verbal fluency ($p < .001$) indicating that fatigue was related to but did not explain group differences in cognitive function. Executive apathy was a significant predictor of all composite scores (executive, social cognitive, verbal fluency; all $p < .05$).

Next, health-related quality of life (HR-QoL) was addressed with a multiple regression model. Fatigue arose as the most significant predictor of HR-QoL in PwHD (Robust HC3 SEs: $\text{std.}\beta = -0.65$, $p_{\text{HC3}} = .004$) with UHDRS Function ($p_{\text{HC3}} = .012$) in an overall model that explained 53% of the variance in HR-QoL, when controlling for functional ability, apathy, depression & SDMT (cognitive processing speed) scores (**Figure 76**):

'HR-QoL' ~ 'UHDRS Function' + 'Depression' + 'DAS Total score' + 'Fatigue' + 'SDMT'

9.3.9 PwHD: Apathy over time

To answer the question if apathy scores change over time, a longitudinal analysis of complete cases was carried out (n : Controls=20; PwHD=24). The groups were matched for age, sex, education and the interval between testing (mean=18.48 months; all $p > .05$; **Table 55**). Linear mixed effects models assessed apathy scores over consecutive timepoints (T1, T2; Level 1) and groups (Control vs. PwHD; Level 2), allowing the intercept to vary by subject (random effects=(1|id)). The interaction of group and timepoint did not significantly improve the models for any apathy domain, nor the testing interval (all $p > .05$) so neither were included in the final models:

$\text{lmer}(\text{'apathy domain'} \sim \text{timepoint} + \text{group} + (1|\text{id}))$

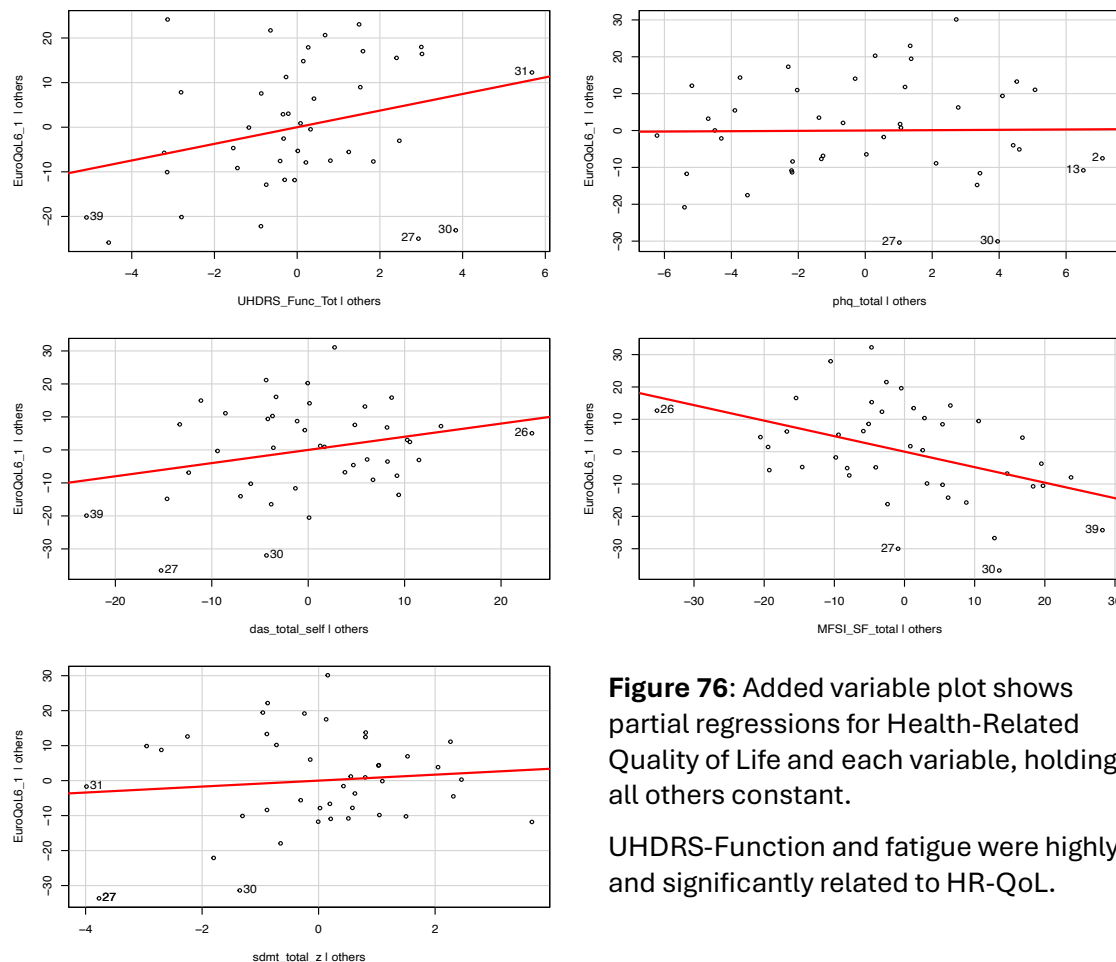


Figure 76: Added variable plot shows partial regressions for Health-Related Quality of Life and each variable, holding all others constant.

UHDRS-Function and fatigue were highly and significantly related to HR-QoL.

There were influential points found in three of the four final models. Sensitivity analyses found these did not alter interpretation and so remained in the final models. Group means and abnormality rates are shown in **Table 56**. Results from the LMM are given in **Appendix 20**. All models showed significant fixed effects for group with the exception of emotional apathy, as expected from findings in previous section. Timepoint was not a significant predictor in any of the models suggesting that although group differences in apathy domain scores were present, scores did not change between timepoints. Large proportions of the variance was explained by random between-subject effects (Intercept SD range=2.83–3.94).

The proportion of variance explained by fixed effects (group and time; Marginal R^2) was low, between 0.9% (Emotional apathy) and 20% (Executive apathy), but which was mostly explained by group membership. Residual (within-subject) variance explained 56%–76% of overall model variance (conditional R^2). The main effect of group on Initiation apathy approached significance, with controls reporting 2.31 points less on average than

the HD group holding time constant and accounting for individual differences ($B = -2.31$ [95% CI: -4.92, 0.30], $t(42) = -1.76$, $\beta = -0.48$, $p = .086$).

Neither timepoint nor the interaction of group and time were significant in explaining any proportion of the variance in apathy scores, suggesting both groups reported consistent levels of apathy over the 18 month interval.

Table 55: Sample for longitudinal apathy analysis. Cases with consecutive and complete DAS scores were included. Manifest and premanifest groups combined into one 'HD' group.

Baseline Characteristics Longitudinal Sample	Controls	HD	p
n	20	24	-
Interval (months) M(SD)	18.6(5.8)	18.3(4.2)	.85
Age M(SD)	45.0(13.1)	49.8(12.1)	.21
Sex (% Female)	60%	58.3%	.91
Premanifest at T1 %	-	75%	-
Converters at T2 (n)	-	3	-
CAG available (n) ^a	-	20	-
CAG repeats ^a	-	41.6(3.1)	-
CAP100 ^{a,b}	-	89.2(25.0)	-
Disease Burden ^{a,c}	-	288.9(114.7)	-
Subgroup clinical characteristics		preHD	manHD
n	-	18	6
UHDRS-Function Md (IQR)	-	25(0)	21(4.3)
UHDRS-Function Range	-	22-25	14-25
UHDRS-TFC Md (IQR)	-	13(0)	10(4.5)

^a 4 HD cases (2 manifest, 2 premanifest) did not have CAG repeat numbers available to calculate Disease burden and CAP100 scores; ^b CAP100³¹⁵; ^c Disease Burden from Penney et al. (1997).³¹²

Table 56: Longitudinal apathy outcomes by group on self-rated DAS for PwHD and controls.

DAS Subtest (Raw Score)	T1		T2	
	Control	PwHD	Control	PwHD
Total	22.20(5.9)	28.9(10.1)	21(8.7)	29.13(12.6)
(% abnormal)	0%	16.70%	5%	20.80%
Executive	4.75(2.6)	9.08(5.4)	4.85(4.1)	9.63(5.5)
(% abnormal)	0%	20.80%	5%	20.80%
Emotional	7.45(2.9)	7.88(4.4)	6.85(2.96)	7.54(4.4)
(% abnormal)	0%	0%	0%	4.20%
Initiation	10.00(3.1)	11.96(4.7)	9.3(4.58)	11.96(5.8)
(% abnormal)	5%	20.80%	5%	29.20%

Table 57: Final linear mixed effects models for apathy domain scores over two time points. HD: n=24; Control: n=20.

DAS Apathy Domains	Marginal R ²	Conditional R ²	Intercept Variance (SD)	B	df	t	β	p
DAS Total (model intercept ^a)	0.13	0.73	8.15	29.24	50.32	15.15	0.35	< .001
Time				-0.43	43	-0.37	-0.04	.71
Group				-7.42	42	-2.72	-0.72	.01
Executive (model intercept ^a)	0.2	0.76	3.87	9.18	50.15	10.06	0.37	< .001
Time				0.34	43	0.63	0.07	.54
Group				-4.55	42	-3.52	-0.89	.001
Emotional (model intercept ^a)	0.009	0.56	2.83	7.94	54.73	10.83	0.13	< .001
Time				-0.46	43	-0.85	-0.12	0.4
Group				-0.56	42	-0.55	-0.15	0.58
Initiation (model intercept ^a)	0.06	0.72	3.94	12.12	49.9	13.1	0.25	< .001
Time				-0.32	43	-0.58	-0.07	.562
Group				-2.31	42	-1.76	-0.48	.086

^a Intercept represent DAS domain score of the PwHD group at T1 as the reference.

9.4 Discussion

In line with the main aims of this thesis, the transdiagnostic relevance of multidimensional apathy in neurodegenerative disorders is presented. This chapter demonstrates that apathy is a common and clinically relevant symptom in both ALS and HD, with distinct domain-specific profiles and meaningful associations with cognitive and functional outcomes. In ALS, carer-rated apathy, particularly Initiation apathy was linked to cognitive and motor symptoms, while in HD, self-rated apathy was highly related to executive dysfunction and depressive symptoms, particularly in the Executive apathy domain. Together, the findings highlight disease-specific patterns and shared mechanisms underlying behavioural and cognitive constructs apathy in neurodegenerative disorders, using neuropsychological and neuroanatomical models as interpretative frameworks. Consistent with the theme of this thesis, the focus is on the pre- and peri-manifest extramotor profile, and is one of the few longitudinal studies of apathy in HD. Here we elaborate upon prior research highlighting the bidirectional relationship and overlapping neuroanatomical mechanisms shared by apathy and cognitive performance in both disorders.

The DAS was developed for use in ALS, aiming to neutralise the confounding effects of reduced motor function on self-reported measures of apathy.⁴³² It has shown utility in other neurodegenerative diseases including AD and FTD^{739,740}, MS⁷⁴¹, as well as HD⁴⁴¹, where it has demonstrated early promise in differentiating fronto-striatal extramotor subtypes. The current studies extend on this work in HD and address gaps in previous work in ALS, analysing function using the DAS, ECAS and BBI in a clinic-based sample of pwALS. Highlighted are the independent and shared effects of these overlapping constructs.

9.4.1 Profiles of apathy

pwALS and PwHD both had significantly elevated levels of Initiation apathy, but the HD group also displayed high rates of Executive apathy by abnormality cut-offs and compared to control subjects. Females were rated as having lower apathy than males in both the HD and the ALS cohorts. Apathy ratings largely agree and show good inter-rater reliability between patients and their caregivers/informants in ALS¹⁵⁴ and HD.^{441,742} This indicates the validity of self- and carer-reported outcomes in these groups, but variation in agreement across the disease-span has been reported on behavioural measures in

HD.³⁷⁸. Not included in this thesis is a small inter-rater reliability study of participants and proxies which examined this directly.

9.4.1.1 *pwALS: apathy profiles*

Consistent with the literature, 33% of pwALS were rated by a carer as having clinically significant Initiation apathy, with only 5% in the abnormal range on Executive and Emotional domains.^{154,743} Although rates of Total apathy are lower (18.4%) than the rates of 30-45% reported in the literature^{152,153,155}, 36.8% of pwALS were impaired on at least one apathy domain at baseline.

9.4.1.2 *pwALS: apathy & general behavioural impairment*

General behavioural symptoms (BBI) were high in this cohort, but concordant with previous studies in ALS which show behavioural impairment in up to 88% of pwALS without FTD.^{152,156,744} Here, 54.9% of patients were rated by a caregiver as having clinically significant behavioural change since symptom onset (mild=39.4%, severe=15.5%). Despite the exclusion of pwALS with FTD diagnoses, 15.5% of patients would meet criteria for ALS-FTD based due to severe behavioural change being noted on the BBI. These rates of abnormality were not reflected in DAS Apathy scores however. General behavioural scores and apathy were related, but rates showed divergence, indicative of broader behavioural phenotype not limited to apathy.

9.4.1.3 *PwHD: apathy profiles*

Overall rates of apathy are similar to those reported in the literature for the early stage manHD (24–76%).^{381,384} 62.5% of the manifest participants and 26.9% of the premanifest participants rated themselves as having clinically significant apathy in at least one of the three DAS subdomains. Both the manifest and premanifest groups endorsed increased levels of self-reported Total and Executive apathy on the DAS, compared to controls. Increased Initiation apathy was evident for manHD also, and was at the trend level after correction for multiple comparisons between controls and preHD. Additionally, the profile of significantly increased rates of abnormal Executive and Initiation apathy is consistent with the literature in manHD⁴⁴¹, but is the first to detect this profile in preHD (**Figure 71**). Atkins et al.⁴⁴¹ did not find elevated DAS domain scores in the premanifest sample, contrary to the current study (Initiation:19.2%; Initiation: 15.4%). Estimates of increased (unidimensional) apathy rates in premanifest PwHD range from 10-33%.³⁸¹ The rates across abnormal domains reported in the current study (15-27%) fall in the middle of this range. Only the manifest group showed significantly elevated

Emotional apathy (i.e.: flattened affect), but to a smaller extent than the other sub-domains (12%). This might suggest a predominating profile of Executive and Initiation apathy, also evident to a milder degree in preHD, which progresses to include Emotional apathy in later stages. This was not supported by linear regression models however.

9.4.2 *PwHD: apathy & depression*

Levels of depression or anxiety were not significantly higher at the group level between preHD and controls, but rates of clinically significant depression (% abnormal) were in both preHD and manHD groups. Depression and anxiety scores were highly correlated in this sample and similar to the rates of apathy. This may suggest generally increased distress in those with elevated scores on any domain. Figures are consistent with studies of early-stage manHD, where prevalence of depression ranges from 30–70% depending on the study.^{233,304,379,384,424} A recent meta-analysis report that the highest rates of depression are in the early manifest stage³⁸⁰ Psychiatric symptoms, including depression vary non-linearly throughout the disease course^{378,424,730}, with up to 50% of Stage 1 HD patients compared to 39% of Stage 5, but conclusions are limited by tool validity in the later stages. Overall, depression pooled frequency for manHD was 38%, and 23% in preHD³⁸⁰, largely equivalent to the rates of mod-severe depression in the current study groups (manHD: 56%; preHD: 23%; controls: 6%).

Higher rates in the absence of significant group differences indicates a sub-group of preHD participants experienced depression, although the majority did not. Previous work in the large multi-centre PREDICT-HD study highlights the increased incidence of depression in preHD relative to controls, but that symptoms are not related to proximity to genetic testing (discovering carrier status), predicted diagnosis or CAG repeat length.⁴²⁴ Not specifically assessed here, suicide risk does increase with proximity to clinical diagnosis.²³¹ Interpretation is challenging, as depression could demonstrate a physiological independence from CAG-associated factors, or reflect more subjective, psychological factors.

Depression scores accounted for a large portion of the variance in DAS Total apathy scores in this study (31%). Hierarchical regression and pairwise comparisons find they are highly related, but distinct in HD, consistent with the diagnostic criteria^{422,429} This effect appears to vary by disease stage however. Depression scores account for Total and Initiation apathy in preHD (approaching significance), but elevated Executive apathy

persists, independent of PHQ-9 scores. This is true for manHD on all domain regression models. Therefore, **Hypothesis 3.2** is partially supported in preHD.

9.4.3 *Apathy & motor function*

Extramotor symptoms of apathy and cognition show some association to motor function or disease progression in both conditions on the ALSFRS-R and UHDRS.

9.4.3.1 **ALS functional measures**

Weak but significant associations between ALSFRS-R scores and both Executive and Initiation apathy subscales contrast those of Radakovic et al.¹⁵⁴, who found no association in pwALS. Here, the BBI and Initiation apathy were independently related to ALSFRS-R scores in multiple regression, in which there was significant shared variance between BBI post scores and Executive apathy. This would suggest that the primary behavioural features of ALS are related to disease progression and motor impairment, at least cross-sectionally at baseline clinic visit. Many studies have found a relationship between extramotor function and disease progression in ALS, but most also have cross-sectional designs, limiting interpretation about longitudinal progression.^{164,745} Whilst a review by Consonni et al.⁷⁴⁶ report differing slopes of decline, suggesting that cognitive/behavioural decline progresses independently of motor deterioration over time.

9.4.3.2 **HD functional measures**

UHDRS Function and TFC were related to self-reported apathy scores, suggesting a connection to functional ability in PwHD. This was only significant in univariate models. Functional ability scores were not associated with apathy when controlling for depression scores (PHQ-9). Cross-sectional analysis found no relationship to CAG-associated disease progression estimates (CAP100) either, supporting previous findings of relative independence of apathy to the disease course of HD.^{424,747} Although functional ability was associated with Total apathy, it showed a stronger cross-sectional relationship with depressive symptoms in this sample. This is in direct contrast with the findings of Atkins et al.(2021)⁴⁴¹ however who report Executive apathy to be uniquely predictive of disease burden, and Emotional apathy of TFC, both when controlling for the effects of age and depression.

9.4.4 Longitudinal apathy profiles in HD

DAS apathy scores showed no deterioration in function over the 18 month study period in the small longitudinal sample. Increased Executive apathy persisted over time in PwHD relative to controls, as did Initiation apathy at the trend level. Matching the cross-sectional apathy profiles shown in the larger group (section 9.4.1.3) and prior studies, apathy appears stable over shorter timeframes in HD.^{730,748} Increasing behavioural symptoms have been recorded across the premanifest stage, with relation to increasing probability of motor diagnosis.³⁷⁸ Whilst apathy is associated with poorer cognitive prognosis³⁸⁷, Thompson et al.'s⁷³⁰ work may be the only demonstration in the literature of longitudinally increasing apathy symptoms, detectable over longer timeframes in later-stage manHD.⁷³⁰ Medication use can often improve the symptoms of depression and apathy, impeding conclusions about the natural disease course.^{223,321,717}

In each domain, there were large subject-specific random effects in the LMMs, indicative of high inter-individual variability in apathy scores. Together with the findings above, it illustrates that some participants in the HD groups experienced high levels of apathy, whilst others did not. Nonetheless, group differences (Control vs PwHD) and profile (Executive and Initiation apathy dominant) remained when controlling for these factors. Whilst the timeframe may not be long enough to demonstrate significant change in this sample, these findings nonetheless contribute to the small body of literature on the longitudinal profile of apathy in the HD.

9.4.5 Apathy & cognition

9.4.5.1 Apathy & cognition in the ALS clinic

At their first clinic visit, 22.8% of pwALS in this large clinic-based sample scored in the abnormal range by age- and education-matched cut-off scores on the ECAS Total score. There were relatively high rates of abnormality on the ALS-non-specific (22.8%) compared to the ALS-specific domain (18.4%). Even considering that FTD cases had been removed, these figures are lower than expected for a large incident ALS population (~35% expected).⁷⁴⁹ However this was a baseline sample, and patients were relatively well (ALSFRS-R Total M(SD)=38.67(5.9)).

There is increasing recognition of memory impairment in ALS⁷⁵⁰, which correlates with hippocampal and parahippocampal atrophy.¹⁸⁷ Lower ECAS memory scores are also associated with Mental fatigue and Executive apathy domain scores in pwALS.⁷⁵¹

Bidirectional models aimed to assess the inference that the constructs progress together in the disease phenotype as well as the impact of apathy on ECAS scores in multivariate models including disease progression. Both apathy and verbal fluency are heavily reliant on the ACC, DLPFC and mPFC.³³² It was hypothesised that the ECAS Fluency domain would be associated with Initiation apathy as a result. Although it approached significance before correction ($p=.051$), these findings do not replicate Radakovic et al.⁷³³ indicating a network overlap using the ECAS. The direction and strength of the effect are consistent however. Overall, ECAS and DAS Total apathy scores were highly associated ($\beta = -0.32$) as were domain-specific associations for the Executive pairing.

The association between DAS Executive and ECAS Executive domain scores contradicting that of Radakovic et al.⁷³³ may be influenced by the broader scale of tests in the ECAS Executive domain. Additionally, Executive apathy appears to be more pronounced in individuals with greater global cognitive impairment in this study, potentially reflecting progression-related deficits in Executive function. The restricted scale range and potential ceiling effects of the ECAS Fluency (0–24) and Social Cognition (0–12) domains may have limited sensitivity to detect such associations. These findings partially support **Hypothesis 3.3**, indicating some overlap in cognitive and behavioural constructs in ALS according to Stuss's model³³², but may have been limited by the constraints of the ECAS, particularly in this relatively cognitively well sample.

9.4.5.1.1 Apathy, cognition & motor function in ALS

To assess the alternate direction, i.e., the effect of apathy on cognitive test performance, multiple and hierarchical regressions demonstrated that DAS Total apathy was a strong predictor of ECAS Total scores, holding all else constant (age, education, onset site, motor function). It had the largest significant independent effect on ECAS Total after onset site in this model, and of a similar magnitude to the model without predictors above ($\beta = -0.30$ & -0.32). This demonstrates the bidirectional effects of ECAS scores on apathy and vice versa, although cannot infer causality.

pwALS with bulbar-onset symptoms scored between 6.2 and 6.9 points lower on the ECAS than spinal and 'other' onset. Depressive scores have been shown to relate to chiefly to social cognition and the executive domain performance on the ECAS, but anxiety was not.⁶⁴⁶

Notably, the BBI was not significant in these models, suggesting a weaker relationship between cognition and general behavioural impairment than apathy alone.

Although behavioural impairment and cognitive impairment often co-occur based on the rates of abnormality (**Table 51**), they can be independent and are not necessarily related.

Conversely, apathy measured by the DAS, did not demonstrate independence statistically and was more likely to co-occur with cognitive impairment (abnormal ECAS Total scores). This is not a perfect relationship however, as 25% of cases did not show agreement between the DAS and the ECAS (i.e.: apathy without cognitive impairment (9.2%) or cognitive impairment without apathy (15.8%)). Although changing the direction does not infer causality, this further illustrates the compounding and inextricable nature of cognitive and behavioural symptoms, especially apathy.

Despite the relationship between cognitive and behavioural measures, cognition was not related to motor function (ALSFRS-R Total score), although Initiation apathy and BBI (general behavioural impairment) were significantly related.

A recent study by Radakovic and colleagues⁷⁴³ report a separate ‘Initiation apathy’ only subgroup, which were less cognitively impaired on the ECAS but who had higher NIV use than other groups and lower ALSFRS-R scores, implying higher physical disability unrelated to other behavioural and cognitive functions. pwALS presenting with higher overall apathy (‘mixed-Emotional apathy’) did have lower cognitive and higher behavioural scores compared to pwALS without apathy in this study. This study controls for the effect of physical disability (ALSFRS-R) scores when examining apathy and caregiver burden, but does not for apathy itself. Nor were there controls for depression, despite higher PHQ-9 scores in the Initiation apathy group and the authors highlighting the importance of doing so in previous work.¹⁵⁴ This again highlights the possibly confounding effects of decreased motor function on carer-reported behavioural measures where more objective measures, which are related, are not associated.

9.4.5.1.2 *The effect of C9orf72 in ALSc/bi*

C9orf72 has been linked with increased rates of cognitive and behavioural symptoms in ALS, as well as an earlier age of onset and more rapid disease course.⁶⁸ In disagreement with most findings, there were no significant differences between C9+ and C9- pwALS on any measure of cognition or apathy, with the exception of ECAS Visuospatial domain in the current sample. The rates of ALSc/bi by the Strong Criteria were also equal across genotypes. This is in contrast the majority of the literature, which describes increased rates of cognitive and behavioural impairment in C9orf72-ALS.⁷⁵² But supported by others showing no differences between C9+ and C9- pwALS on the ECAS.⁷⁵³ Only clinical genetic data were available (46.7% of total sample), which is biased towards

clinical judgement, and who should be sent for testing. Selection bias means this sample of genotyped participants is likely not reflective of the ALS population as a whole, which may reduce the generalisability of these results.

9.4.5.2 Apathy & HD-Cognition

Consistent with the cross-sectional HD-Cognition study findings, manHD had significantly lower scores than controls in each cognitive composite (general, executive, social cognition) and test employed (FAS). Trends were evident for the preHD group also consistent with **Chapter 8**, but were not significant in this smaller sample when controlling for TOPF-FSIQ, after correction for multiple comparisons at baseline. Previous chapters detail the cross-sectional and longitudinal trends in cognition. The focus for this section is the cognitive correlates of apathy.

The current analyses found the executive composite and phonemic verbal fluency scores were predictive of Executive and Initiation apathy scores respectively. Higher scores on the cognitive tests were associated with lower apathy scores in PwHD, even when controlling baseline premorbid IQ scores, provide supporting evidence for **Hypothesis 3.3**. The general and social cognitive composite scores were not predictive of their corresponding apathy domains however (DAS Total and Emotional respectively). While error is inherent in combining variables as a composite, this would imply that general cognitive function does not decline in concert with increasing overall apathy. This is in contrast to many studies which found associations between apathy and SDMT scores, which is predictive of pervasive apathy in HD.⁷⁴⁸ It has also been shown to predict a more rapid cognitive decline in preHD, with motor decline becoming more predictive of cognition after motor symptom onset.³⁸⁷

Despite decreased social cognitive function in manHD, the aspect of **Hypothesis 3.3**, that shared orbitofrontal-striatal network dysfunction would cause both decreased ToM and increased Emotional Apathy, was not supported. Floor-effects on the Emotional apathy DAS subdomain may explain this result. Though present for manHD, group differences were much smaller for Emotional apathy. Two recent studies did find apathy and affect recognition are associated in HD, highlighting this hypothesised mechanistic link within the Dimensional Apathy Framework.^{500,735} Both treat apathy as a unitary construct, without differentiating sub-types. This method confers broader extramotor associations that may not differentiate neuroanatomically, as is the goal here.

9.4.6 PwHD: Fatigue, apathy & cognition

Fatigue (total) was strongly related the Executive apathy subdomain of the DAS. Mental fatigue in particular was the most commonly elevated domain associated with cognitive, functional and quality of life variables, but neither fatigue nor apathy domain scores accounted for significant portion of group differences in cognition. Group differences in cognitive function remained for preHD and manHD, even when apathy and fatigue were accounted for in speed of processing, and for the manHD group for executive function (CWIT), social cognition and verbal fluency tasks, supporting **Hypothesis 3.4**. Fatigue also arose as the most significant predictor of HR-QoL in the PwHD group with a moderate-large effect, more so than functional ability, apathy, depression or cognitive scores (SDMT). Fatigue's independent impact on HR-QoL could be the target of interventions to improve patient QoL.

Together the apathy profiles observed in these studies are consistent with neuroanatomical models upon which the hypotheses for this chapter were based. Findings support **Hypothesis 3.1**, which expected that superior-medial-striatal circuit is primarily diminished in pwALS, presenting behaviourally as increased Initiation apathy. In addition to evidence of dysfunction in these circuits, the HD profile was hypothesised to also present with increased Executive apathy due to co-occurring dorsolateral-striatal circuit dysfunction. Not hypothesised was that Emotional apathy would also be increased, as it is here, albeit to a lesser degree, implicating secondarily the orbitofrontal-striatal circuits. This is consistent with neuropathological evidence of primary striatal degeneration in HD causing dysfunction in all three primary fronto-striatal circuits, and concordant with social cognitive deficits, commonly presenting in HD.

9.4.7 Study-specific limitations

Mild-to-severe depressive symptoms were high in this sample of controls—30.3% and 24.24% of depression and anxiety respectively. Rates of depression and anxiety are relatively high in the general population, approximately 5-15%.⁷⁵⁴⁻⁷⁵⁶ Rates in controls in the current study may be higher due to the inclusion of Gene Negative and unrelated HD family members in our control sample. Whilst maximising the ecological validity of the pathological CAG-associated conclusions, this method could obscure more subtle trends in PwHDs where the control group is not reflective of the general population.

In HD-Cognition, there was homogeneity of scores in the control group relative to the heterogeneity of scores within PwHD groups. This was addressed by applying robust HC3 standard errors to account for potential non-constant variance in residuals. This did

not change the direction of the findings, but may have affected the strength of the effects. The UHDRS and ALSRFS-R functional scales also have limitations to sensitivity, especially for preHD where functional changes are not expected. Sample size did not allow for random slopes in the mixed models which could better estimate the interactions of CAG, age, functional scores and cognitive/behavioural symptoms.

9.5 Conclusion

This chapter explored various aspects of multidimensional apathy in ALS and HD, providing transdiagnostic insights into its clinical relevance, cognitive correlates, and relationship to functional decline. Apathy was common in both diseases, with Initiation apathy emerging as a shared and clinically significant feature, while Executive apathy was more pronounced in HD. The overall profiles aligned with established fronto-striatal circuit models and supported the distinct but overlapping cognitive behavioural features of these disorders.

Importantly, premanifest participants also displayed clinically significant levels of apathy, underscoring its presence in the prodromal phase and highlighting the need for early detection and extramotor diagnostic criteria. Across both cohorts, cognitive performance was associated with specific apathy domains, supporting the hypothesis that apathy subtypes map onto distinct cognitive profiles and fronto-subcortical circuits, and partially replicating the findings of Radakovic et al. (2017)⁷³³ in this large incident, clinic-based ALS sample.

Executive function and verbal fluency were consistently associated with Executive and Initiation apathy domains respectively, while general and social cognition showed weaker links. Depression, though strongly related to apathy in HD, did not fully account for group differences, reinforcing their conceptual and neurobiological distinction. The bidirectional nature of apathy and cognition highlights how motivational and disease-related network abnormalities can compound to affect cognitive outcomes.

Although apathy scores remained stable over 18 months in the HD cohort, significant between-group effects persisted, and individual variability was high. Together, these findings demonstrate that apathy is not merely a by-product of global disease progression, but rather a semi-independent feature with unique clinical, cognitive, and functional correlates. The findings support **Hypotheses 3.1–3.4** and contribute to a growing body of literature advocating for the use of validated apathy subscales to track disease features across conditions to elucidate mechanistic links.

10 Chapter 10:

Results: a cognitive screening tool for HD – ECAS-HD

10.1 Introduction

10.1.1 *Cognitive screening tools*

Recommendations from the HSG Neuropsychology Working Group³¹⁰ outline the importance of routine screening for cognitive and behavioural symptoms in HD. However, full neuropsychological assessments are labour- and time-intensive and may not be suitable or feasible for all patients. There are no cognitive screening tools specifically designed for HD-related cognitive impairment.

Traditional screening tools such as the MMSE and MoCA are widely used, but lack sensitivity to early and domain-specific impairments in HD and are poorly suited for serial monitoring.⁷⁵⁷⁻⁷⁶³ Others have employed Parkinson's disease cognitive screening tools (PD-CRS) in HD⁷⁶⁰, which demonstrated high sensitivity/specificity against the MMSE, but does not have repeatable forms, impacting reliability for serial assessment to monitor function over time.

The RBANS is another cognitive battery that has shown utility in HD-related cognitive impairment.^{338,343,764} It is midway between that of the full neuropsychological assessment and a cognitive screening tool, though may not be feasible to carry out as routine screening measure in an outpatients clinic. A brief, domain-specific and repeatable cognitive screening tool is needed for use in HD.⁷⁶⁵

10.1.2 *The ECAS*

Cognitive changes observed in HD overlap considerably with the domains captured by the ECAS, matching the majority of the 'Recommended' domains of the HSG

position paper for the neuropsychological assessment of HD.³¹⁰ It takes approximately 15-20 minutes to administer, not only acting as a cognitive screening tool for detection, but can also quantify the magnitude and characterise the nature of cognitive impairment by domain, albeit with limited sensitivity and specificity. Unlike other screening tools, the breadth of the ECAS domains allow for monitoring of domain-specific function. Published normative cut-offs for different categories of age and education improve sensitivity.^{162,163}

The ECAS boasts strong diagnostic accuracy for ALSci, and is now routinely used in both clinical care and clinical trials.^{16,52,159,161-163} It is designed to be accessible to patients with motor speech or limb impairments, making it suitable for conditions with progressive motor decline. It has also been validated in multiple languages and cultural contexts, including Ireland^{162,163}, Swiss-German⁷⁶⁶, Italian⁷⁶⁷, Chinese⁷⁶⁸, Dutch⁶¹⁶, Norwegian⁷⁶⁹, Swedish, Polish, German⁷⁷⁰, Russian⁷⁷¹, Egyptian⁷⁷² and Greek.⁷⁷³

Discriminatory ability across diagnostic categories has already been shown in other neurodegenerative disorders, effectively distinguishing between pwALS and those with AD⁷⁷³, bvFTD and AD⁷⁷⁴, and PD and PSP.⁷⁷⁵ A small study by Carelli et al. (2021)⁷⁷⁶ demonstrated the efficacy of an Italian version of the ECAS in manifest PwHD compared to patients with ALS and PD. PwHD showed lower scores across the ECAS domains, compared to pwPD and pwALS. The ALS-non-specific domains, specifically memory was most strongly impaired. They did not compare performance to the gold-standard neuropsychological assessment however, but to the MoCA, RMET and Frontal Assessment Battery. Whilst these are validated and standardised tests, validation against the gold-standard in HD is necessary. Neither has the ECAS been evaluated for sensitivity in prodromal PwHD, nor of the performance of serial administration of alternate forms over time.

10.1.3 Chapter aims

This chapter addresses **Aim 8; Hypotheses 8.1 & 8.2**

The objective of which is to evaluate the diagnostic and predictive ability of the ECAS for detecting cognitive dysfunction in premanifest and early manifest HD. Validation of ECAS subtests against an extended neuropsychological battery will be followed by evaluation of performance over time on serial administration of alternate forms (A and B).

10.2 Methods

10.2.1 *Study Design*

This was a cross-sectional and longitudinal validation study designed to assess the convergent validity and diagnostic utility of the ECAS in HD. Procedures were adapted from published validation studies in ALS populations in Ireland^{162,163}, and addressed in the methods section.

10.2.2 *Participants*

Participants in the HD-Cognition study completed the ECAS alongside the extended neuropsychological battery. Recruitment and other procedures are consistent with the main study procedures outlined in the methods section. The control group included genetically confirmed Gene Negative HD-family members, non-biological family controls (i.e.: spouses) and healthy controls. Controls were age- and education-matched to the premanifest group. Exclusions matched the cross-sectional HD-Cognition study findings. Baseline data are available for 37 controls, 38 premanifest PwHD and 14 manifest PwHD. Follow-up (T2) data were available for 25 controls and 38 PwHD participants. Of these, six individuals initially classified as premanifest progressed to manifest HD by T2. Therefore PwHD were grouped together for longitudinal analyses, and stratified by CAP100 score. Participant demographic data are presented in **Table 59**. Sample descriptives of those not completing T2 are shown in **Appendix 24**.

10.2.3 *Assessment Procedures*

Neuropsychological assessments were performed in accordance with HD-Cognition study procedures described in the methods section.

10.2.3.1 *ECAS*

ECAS A was administered at baseline, and ECAS B at T2 follow up. The ECAS and extended neuropsychological battery were usually administered in one appointment, although a minority participants completed it over two sessions, within one week. The two main domains in the ECAS, ALS-Specific and ALS-Non-Specific distinguish cognitive subtypes valid for ALS patients, the names of which may not be meaningful for HD. Despite this, domain names are maintained for descriptive purposes.

10.2.3.2 Extended neuropsychological assessment

Each ECAS subtest and domain was matched with an analogous measure and composite from the extended battery to evaluate convergent validity, and diagnostic accuracy against the gold-standard assessment (**Table 58**). The selection of scores for each composite was guided by the same domain structure used in the ECAS, and based on Pinto-Grau et al.'s¹⁶³ original validation.

VFI were converted to z-scores from locally generative normative data.¹⁶³ Domain composites were created by summing the z-score for each extended battery task, divided by the number of tests in the respective ECAS domain, ensuring equal weighting.

Table 58: ECAS and corresponding extended neuropsychological test battery domains and tasks.

ECAS Domains	Cognitive domain	ECAS Subtest	HD-Cog Subtest equivalent
ALS-specific			
Language	Naming	Naming	Sydney Language Battery - Naming
	Comprehension	Comprehension	Sydney Language Battery - Comprehension
	Spelling	Spelling	Sydney Language Battery – Comprehension
Verbal Fluency	Free Fluency	Fluency Letter S	FAS VFI
	Restricted Fluency	Fluency Letter T	Letter C - 4 letter words
Executive Function	Working Memory	Reverse Digit Span	Digit Span Backwards WAIS-IV
		Alternation	Digit Span Backwards WAIS-IV
	Inhibitory Control	Sentence Completion	D-KEFS CWIT Inhibition time
	Social Cognition	Social Cognition	Reading the mind in the Eyes
ALS-non-specific			
Memory	Immediate Recall	Immediate Recall	Logical Memory WMS-III - Immediate Recall
	Percent Retention	Delayed Retention	Logical Memory WMS-III - Percent Retention
	Recognition	Delayed Recognition	Logical Memory WMS-III - Recognition
Visuospatial		Dot Counting	RCFT Copy
		Cube Counting	
		Number Location	

10.2.4 Statistics

10.2.4.1 Validation

Published age and education cut-off scores are used to determine abnormality for the ECAS domains.^{162,163} For the extended neuropsychological battery, abnormality was

defined as a z-score ≤ -2 (2SD below the control mean) to correspond with ECAS classification thresholds.¹⁶³ Rates of impairment are reported.

Pearson correlations were computed to evaluate convergent validity between ECAS subtests and domains, and their extended battery test/composite score equivalents. Diagnostic accuracy was assessed via sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with the extended battery abnormality classifications as the reference standard.⁶¹⁶ Where poor accuracy is provided by existing cut-offs, receiver operating characteristic (ROC) curve analyses were used to determine optimal classification thresholds determined by Youden's J using the 'pROC' package in R.⁶¹⁷ Optimal threshold, sensitivity, specificity and area under the curve (AUC) are reported. p-values were adjusted for multiple comparisons using the Benjamini-Hochberg correction across all tests and domains.

10.2.4.2 ECAS performance over time

For the sub-group who completed follow-up assessment and completed ECAS B, mean change scores and change status (Decline, No Change, Improved) based on published Reliable Change Indices (RCI)¹⁶² were calculated to illustrate clinical utility at the individual level.

For group longitudinal analyses, LMMs were fitted using the 'lme4' package in R.⁶⁰⁴ The models examined ECAS scores between consecutive timepoints (T1, T2; Level 1) and groups (Control vs. PwHD; Level 2) They assessed changes in ECAS scores over time, with timepoint (T1, T2) and group at baseline as fixed effects, and a random intercept for each subject to account for within-subject correlations. The interaction of group and time was also included ('group T1' $\times$ timepoint). Additional covariates were included as appropriate. For PwHD participants with available CAG-repeat data (n=41), CAP100 was included in separate models to assess its association with ECAS performance.

Equation 14: LMM for ECAS over time.

$$\text{lmer}(\text{'ECAS domain'} \sim \text{'group T1'} \times \text{timepoint} + \text{covariates} + (1 \mid \text{id}))$$

where group is group at baseline, and (1 | id) is the random effects, within-subjects intercept

Table 59: Demographic characteristics of the ECAS Validation Sample

	ECAS A: T1			F(df)/H χ^2	p	ECAS B: T2			F(df)/H χ^2	p
	Controls	PreHD	Manifest			Controls	PreHD	Manifest		
n	37	38	14			25	23	15 ^a		
Age	46.78 (11.96)	45.29 (11.34)	53.5 (14.6)	2.38 (2,86)	.099	48.76 (12.15)	45.96 (10.56)	55.87 (10.6)	3.61 (2,60)	.033*
Sex (% Female)	62.2%	65.8%	28.6%	6 (2)	.05	60%	60.9%	53.3%	0.2 (2)	.9
Years Education	17.15 (2.86)	16.47 (2.84)	15.43 (3)	1.88 (2,86)	.159	17.54 (3.26)	16.96 (2.67)	15.2 (2.2)	3.27 (2,60)	.045*
TOPF-FSIQ (T1)	111.22 (10.78)	102.85 (11.44)	95.99 (10.9)	10.5 (2,86)	<.001*	112.16 (9.46)	104.58 (12.33)	99.81 (10.58)	6.44 (2,59)	.003*
CAG available (n) ^b		32	9				21	10		
CAG		41.94 (2.48)	44.11 (4.3)				41.05 (1.5)	43.3 (3.9)		
CAG (range)										
CAP100 ^b		84.11 (21.93)	109.77 (17.3)				81.48 (22.53)	114.47 (16.7)		
Disease Burden ^b		280.73 (92.83)	403.44 (103.4)				257.02 (92.2)	408.05 (100.8)		
Ttdx ^b		9.28 (13.35)	-4.11 (10.5)				11.48 (13.86)	-6.5 (9.9)		
UHDRS-Function Md (IQR)		13 (0)	9.5 (5.3)				13 (0)	10 (4.5)		
UHDRS-Function Range		19 – 25	8 – 25				22–25	6–24		
UHDRS-TFC Md (IQR)		25 (0)	19 (8.8)				25 (0)	19 (6)		
UHDRS-TFC Range		5 – 13	3 – 13				11–13	3–13		
UHDRS-Ind. Md (IQR)		100 (0)	80 (25)				100 (0)	80 (25)		

Scores presented as Mean(SD), unless otherwise stated. * denotes statistical significance. Ttdx=Time to predicted diagnosis by Langbehn model.

^a n=6 premanifest PwHD at T1 received clinical diagnoses between timepoints and were considered manifest HD at T2.

^b CAG-repeat length data availability from clinical genetics report. CAP100, disease burden & ttdx scores only available for these participants.

^c UHDRS-Ind=Independence

10.3 Results

10.3.1 *Demographic group comparisons*

Table 59 shows participant demographic data. Groups did not differ significantly in terms of age, education or sex distribution (all $p \geq .05$). The manifest group tended to be older, have lower education and have fewer females at baseline, but these did not reach significance. Group comparisons for T2 showed significant differences between the groups, for age ($F(2,60)=3.61, p=.033$) and education ($F(2,60)=3.27, p=.045$) driven by the manifest group, who were significantly older and less educated than controls and premanifest participants (all $p < .05$). Control and premanifest groups did not differ ($p > .05$).

10.3.2 *Convergent validity: ECAS A – T1*

Pearson correlation coefficients were statistically significant for all paired ECAS A subtests and extended battery composite scores (all $p_{\text{adj}} \leq .014$; **Table 60**), supporting the construct validity of the ECAS in this population. Effect size was smallest for DSB and ECAS Alternation ($r = 0.293, p_{\text{adj}} = .014$) and the highest was for FAS VFI and the ECAS Fluency domain ($r = 0.868, p_{\text{adj}} < .001$).

10.3.2.1 *ALS-Specific Domains*

In the Language domain, ECAS subtests showed a moderate correlation with all Sydney language battery subtests ($p \leq .003$). As with free verbal fluency, Restricted Fluency showed a large association ($r = 0.619, p_{\text{adj}} < .001$).

In the Executive domain, the small correlation between DSB the ECAS Alternation subtest, may be expected, as these tasks are similar, but not necessarily equivalent tasks. Nonetheless, a more direct match was not available, and this effect was statistically significant ($r = 0.293, p = .014$). All other Executive subtests showed moderate-to-large convergence ($p < .001$).

10.3.2.2 *ALS Non-Specific Domains*

All ECAS Memory domain tasks displayed convergence with large effect sizes with the extended battery tasks and composites (all $r > 0.5, p_{\text{adj}} < .001$). Visuospatial performance for the three ECAS tasks were taken together, and so was equivalent to the Visuospatial domain score. They showed a moderate correlation with the Rey Complex Figure Test (RCFT) Copy trial ($p_{\text{adj}} < .001$).

10.3.2.3 ECAS Domain & Composite Scores

Strong and statistically significant correlations were observed between ECAS composite scores and all HD-Cognition composite equivalents (**Table 60**). The ECAS Total score displayed the strongest convergence ($r=0.857$, $p_{\text{adj}} < .001$).

Table 60: Convergent validity of ECAS and HD-Cognition subtests: ECAS A – T1.

ECAS Domains	ECAS Subtest	HD-Cog equivalent		
ALS Specific			<i>r</i>	<i>p.adj</i>
Language	Naming	SLB – Naming	0.479	<.001
	Comprehension	SLB – Comprehension	0.453	<.001
	Spelling	SLB – Comprehension	0.375	.003
Verbal Fluency	Free Fluency	FAS vfi	0.868	<.001
	Restricted fluency	Restricted Letter C vfi	0.619	<.001
Executive Function	Reverse Digit Span	DSB	0.766	<.001
	Alternation	DSB	0.293	.014
	Sentence Completion	CWIT Inhibition time	0.314	.003
	Social Cognition	RMET	0.422	<.001
ALS Non-Specific				
Memory	Immediate Recall	LM Immediate Recall	0.757	<.001
	Delayed Retention	LM Percent Retention	0.54	<.001
	Delayed Recognition	LM Recognition	0.526	<.001
Visuospatial	Dot Counting	RCFT Copy	0.497	<.001
	Cube Counting			
	Number Location			
Composite scores			<i>r</i>	<i>p.adj</i>
Total			0.857	<.001
ALS-Specific			0.811	<.001
Language			0.584	<.001
Verbal Fluency			0.804	<.001
Executive Function			0.684	<.001
ALS-Non-Specific			0.705	<.001
Memory			0.734	<.001
Visuospatial			0.497	<.001

SLB=Sydney Language Battery, vfi=Verbal Fluency Index. p_{adj} =adjusted for multiple comparison with BH FDR correction.

10.3.3 Sensitivity, specificity & predictive values: ECAS A – T1

Using existing age- and education-matched cut-offs, ECAS Total score demonstrated very high sensitivity and specificity. A very strong negative predictive value (NPV=0.986) indicates that it reliably rules out cognitive impairment in individuals who screen negative. The ALS-Specific composite reached perfect sensitivity and NPV (1.00), though its low PPV (0.333) suggests frequent false positives. The Non-Specific composite

offered moderate sensitivity and high specificity (0.871), and a good balance of PPV and NPV (**Table 61**).

Among domains, Fluency also maintained perfect sensitivity and NPV, while the Executive domain showed high specificity (0.918) but limited sensitivity (0.333) and very low PPV (0.125). Language and Memory demonstrated moderate sensitivity and high NPVs (>0.94), while Visuospatial function remained poorly sensitive (0.238) despite perfect specificity and PPV (**Table 61**).

Table 61: Sensitivity and specificity analysis: ECAS A – T1.

Domains	Sensitivity	Specificity	PPV	NPV
ECAS Total	0.909	0.909	0.588	0.986
ALS-Specific	1	0.854	0.333	1
Language	0.556	0.904	0.417	0.943
Fluency	1	0.848	0.231	1
Executive	0.333	0.918	0.125	0.975
Non-Specific	0.6	0.871	0.5	0.91
Memory	0.857	0.885	0.4	0.986
Visuospatial	0.238	1	1	0.797

10.3.4 ROC analysis: ECAS A – T1

Given the poor sensitivity and PPV for the Executive domain at the fixed cut-off, it might not be optimal for this sample. An ROC analysis was conducted which found a threshold of 31.5 (optimal Executive domain score) had a sensitivity and specificity of 0.83 and 0.88 respectively (AUC=0.875). This greatly improves classification accuracy. ROC curves are also shown in **Figure 77** for ECAS Total, ALS-specific and ALS-non-specific domains, with optimal cut-off threshold scores, sensitivity, specificity, and AUC values for each.

10.3.5 Rates of impairment: ECAS A – T1

The ECAS shows a consistent level of over-sensitivity across the domains, with the exception of the Visuospatial domains, which shows high rates of abnormality for the RCFT Copy trial (**Appendix 24**).

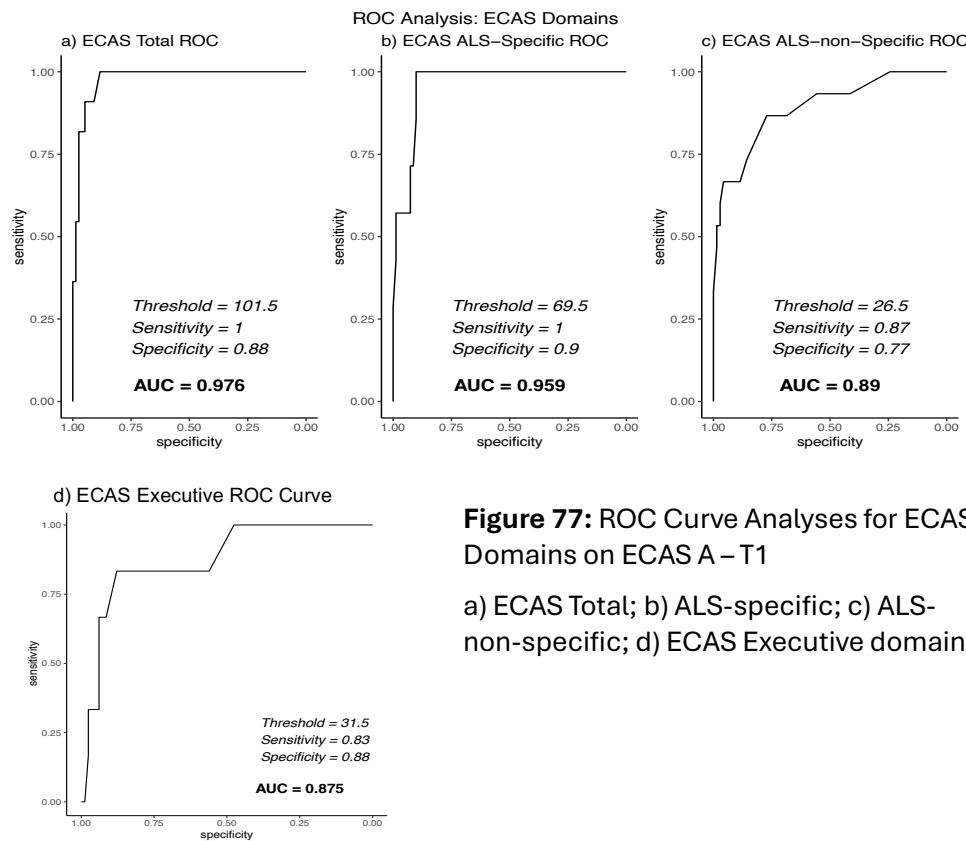


Table 62: Rates of impairment on ECAS and extended neuropsychological test domains ECAS A – T1 by standard normative data.

ECAS A	Control		Premanifest		Manifest	
Domains	ECAS	FB	ECAS	FB	ECAS	FB
n	36		38		14	
ECAS Total	0.0%	0.0%	15.8%	5.3%	78.6%	64.3%
ALS-Specific	5.6%	0.0%	13.2%	0.0%	78.6%	50.0%
Language	0.0%	0.0%	14.7%	5.9%	53.8%	53.8%
Fluency	3.3%	0.0%	17.9%	0.0%	63.6%	27.3%
Executive	0.0%	0.0%	7.9%	2.6%	35.7%	35.7%
Non-Specific	2.9%	5.7%	26.3%	10.5%	58.3%	75.0%
Memory	2.9%	0.0%	18.4%	2.6%	58.3%	50.0%
Visuospatial	0.0%	8.6%	5.3%	21.1%	27.3%	90.9%

FB=extended neuropsychological assessment.

10.3.6 Convergent validity: ECAS B – T2

ECAS B also demonstrated excellent convergent validity, as all subtests were statistically significantly correlated with corresponding extended battery subtests (**Table 63**). Strong effects were observed, highest again for the ECAS Fluency domain ($r = 0.903$), Reverse Digit Span ($r = 0.783$), and Memory (Immediate Recall : $r = 0.762$). The weakest

correlations were for the ECAS Social cognition, ($r = 0.294$, $p_{\text{adj}} = .02$) and ECAS Sentence Completion ($r = 0.382$, $p_{\text{adj}} = .002$), but were nonetheless statistically significant.

Composite domain scores also showed excellent convergence for ECAS Total ($r = 0.84$), ALS-Specific ($r = 0.88$) and Verbal Fluency ($r = 0.87$), with smaller, but still large effects for the ALS-non-specific domain ($r = 0.54$). The Visuospatial domain was the lowest, with weak but significant correlation to the full battery composite ($r = 0.302$, $p_{\text{adj}} = .02$). All others demonstrated strong convergence.

10.3.7 *Sensitivity, specificity & predictive values: ECAS B – T2*

All domain scores demonstrated good to excellent sensitivity and specificity, with the exception of visuospatial domain (see **Table 64**). ECAS B had good overall sensitivity (Total: 0.714) and high specificity and NPV values. ALS-Specific and Language domains showed high sensitivity (≥ 0.875) and specificity (> 0.92), and the Fluency and Memory domains achieved perfect sensitivity and NPVs (1.00). In contrast, the Visuospatial domain showed very poor sensitivity (0.143) despite strong specificity (0.956). PPVs were lowest for the Executive and Total domains ($\text{PPV} < 0.43$), suggesting there were a number of false-positives, although the NPV was excellent for both ($\text{NPV} > 0.96$).

10.3.8 *ROC Analysis: ECAS B – T2*

Although diagnostic accuracy was higher on ECAS B, for consistency, the ROC analysis was performed shown in **Figure 78** for the ECAS domains. A cut-off of 107.5 on the ECAS Total improves sensitivity from 0.71 to 1.0, dropping specificity marginally from 0.87 to 0.78, for an AUC of 0.95. Other domains remain largely unchanged.

10.3.9 *Rates of impairment: ECAS B – T2*

ECAS B had consistently higher rates of impairment relative to the extended neuropsychological battery of tests (FB) across domains and groups, with the exception of visuospatial domain (see **Table 65**). Visuospatial impairment was under-identified by the ECAS (25%) compared to the full battery (66.7%) in the manifest group. 16.7% of controls were below the cut-off, nearly double that of the premanifest PwHD. These patterns were also evident on ECAS A.

For manifest HD participants, impairment rates were highest on Language (64.3% ECAS vs. 42.9% FB), Memory (64.3% ECAS vs. 35.7% FB), and ALS-Specific (60% ECAS vs. 40% FB) domains. Fluency and Executive impairments were moderately detected (40–

53%) across both tools. Premanifest group had lower rates across all domains, from 4.3% to 9.1% on the extended battery, and 8.7-17.4% on the ECAS.

Table 63: Convergent validity of ECAS and HD-Cognition subtests: ECAS B – T2.

ECAS Domains	ECAS Subtest	HD-Cog equivalent		
ALS Specific			r	p.adj
Language	Naming	SLB – Naming	0.572	<.001
	Comprehension	SLB – Comprehension	0.351	.01
	Spelling	SLB – Comprehension ^a	0.427	.001
Verbal Fluency	Free Fluency	FAS vfi	0.903	<.001
	Restricted fluency	Restricted Letter C vfi	0.73	<.001
Executive Function	Reverse Digit Span	DSB	0.783	<.001
	Alternation	DSB	0.551	<.001
	Sentence Completion	CWIT Inhibition time	0.382	.002
	Social Cognition	RMET	0.294	.02
ALS Non-Specific				
Memory	Immediate Recall	LM Immediate Recall	0.762	<.001
	Delayed Retention	LM Percent Retention	0.445	<.001
	Delayed Recognition	LM Recognition	0.556	<.001
Visuospatial	Dot Counting	RCFT Copy	0.302	.02
	Cube Counting			
	Number Location			
Composite scores				
Total			0.842	<.001
ALS-Specific			0.882	<.001
Language			0.719	<.001
Verbal Fluency			0.872	<.001
Executive Function			0.739	<.001
ALS-Non-Specific			0.535	<.001
Memory			0.784	<.001
Visuospatial			0.302	0.02

SLB=Sydney Language Battery, vfi=Verbal Fluency Index. p.adj=adjusted for multiple comparison with BH FDR correction.

Table 64: Sensitivity and specificity analysis: ECAS B – T2.

Domains	Sensitivity	Specificity	PPV	NPV
ECAS Total	0.714	0.873	0.417	0.96
ALS-Specific	0.875	0.926	0.636	0.98
Language	0.875	0.962	0.778	0.981
Fluency	1	0.927	0.636	1
Executive	0.8	0.877	0.364	0.98
Non-Specific	0.75	0.849	0.429	0.957
Memory	1	0.873	0.462	1
Visuospatial	0.143	0.956	0.5	0.782

Figure 78: ROC curve analysis for ECAS domains: ECAS B - T2.

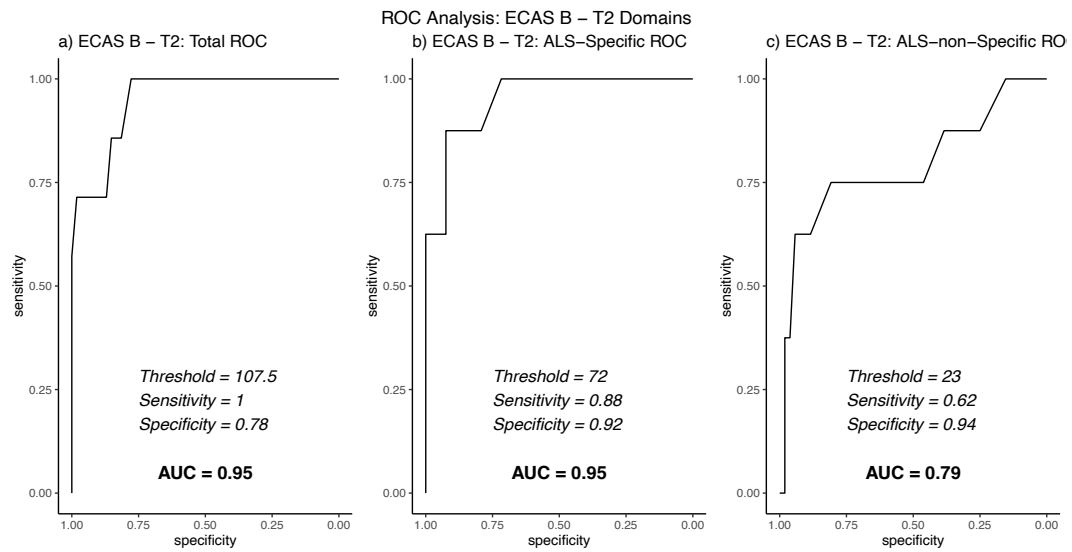


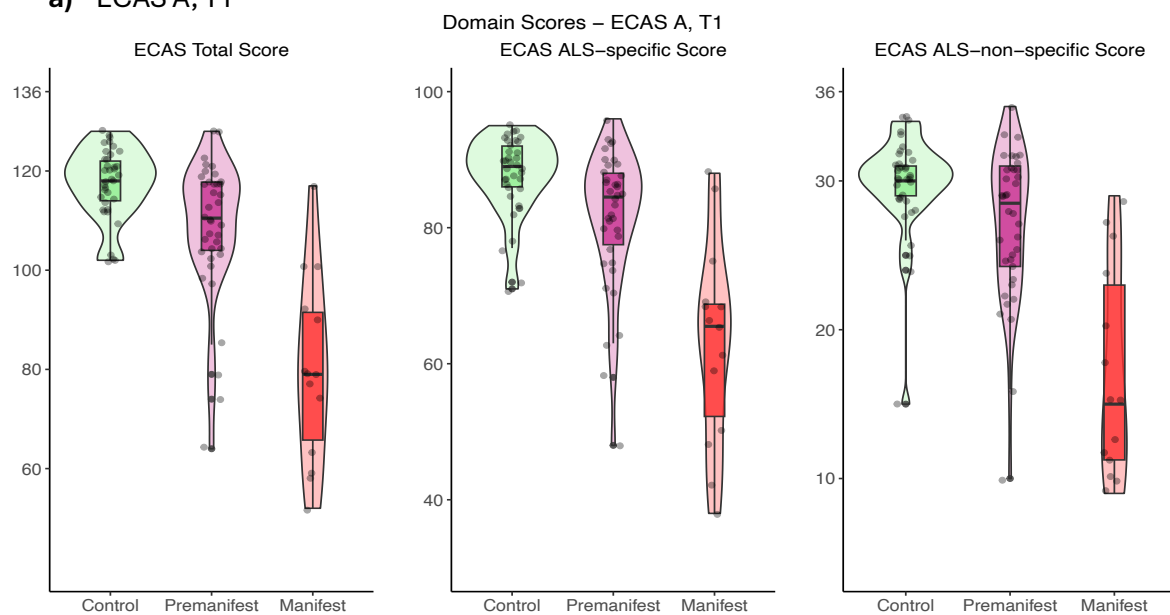
Table 65: Rates of impairment on ECAS and extended neuropsychological test domains ECAS B, T2.

ECAS B T2	Control		Premanifest		Manifest	
Domains	ECAS	FB	ECAS	FB	ECAS	FB
n	24		23 ^a		15 ^a	
ECAS Total	0%	0%	8.70%	4.30%	66.70%	40%
ALS-Specific	0%	0%	8.70%	8.70%	60%	40%
Language	4.20%	0%	17.40%	8.70%	64.30%	42.90%
Fluency	0%	0%	13.60%	9.10%	40%	40%
Executive	0%	0%	13%	8.70%	53.30%	33.30%
Non-Specific	8.30%	0%	4.30%	4.30%	53.30%	26.70%
Memory	0%	0%	17.40%	4.30%	64.30%	35.70%
Visuospatial	0%	16.70%	4.30%	8.70%	25%	66.70%

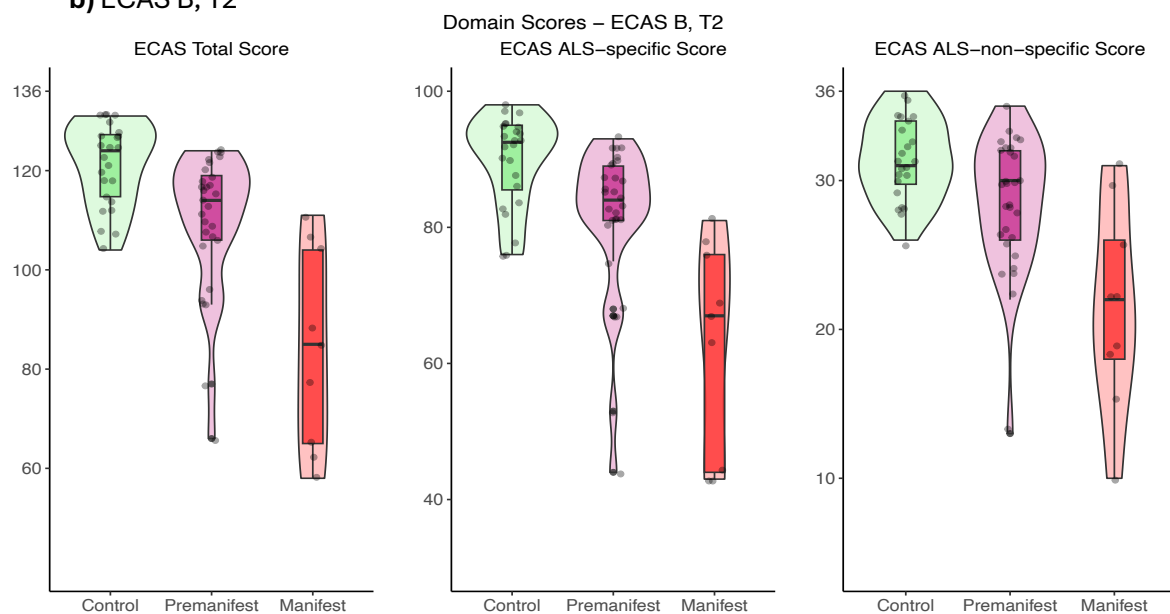
^a n=6 premanifest PwHD at T1 received clinical diagnoses between timepoints and were considered manifest HD at T2.

Figure 79: Violin plots of ECAS scores in validation sample.

a) ECAS A, T1



b) ECAS B, T2



NOTE: a) ECAS A - T1, b) ECAS B - T2. Groups at baseline.

10.4 Results part 2: Longitudinal ECAS performance

Table 66 displays longitudinal sample characteristics and ECAS change scores for controls and PwHD (manifest and premanifest combined).

10.4.1 *Reliable change T1-T2*

21.1% of PwHD declined by the Reliable Change Index (RCI) on ECAS Total and ECAS ALS-Specific, and 10.5% on the non-specific domain. Although the RCI factors in practice effects, and improvements would not be expected across any group, a number of cases improved significantly by the RCI, particularly in the non-specific domain (18.4%; **Table 67**).

Table 66: ECAS domain scores at T1 and T2, change scores (T2 – T1) and demographic variables for longitudinal sample.

	T1 – A		T2 – B		Mean (SD) Δ Scores	
	Controls	PwHD	Controls	PwHD	Controls	PwHD
n	37	52	24	38	24	38
Interval (months; Md[IQR])	-	-	19.7 [9.37]	21.4 [12.7]	-	-
Age	46.78 (11.96)	47.5 (12.7)	48.42 (12.28)	49.87 (11.52)	-	-
Years education	17.15 (2.86)	16.19 (2.89)	17.48 (3.31)	16.26 (2.63)	-	-
CAP100 ^a	-	89.74 (23.41)	-	92.12 (25.83)	-	-
TOPF-FSIQ (T1)	111.22 (10.78)	101 (11.6)	112.16 (9.46)	102.7 (11.76)	-	-
ECAS Total	117.43 (7.2)	100.94 (19.93)	121.25 (8.36)	103.47 (18.87)	1.96 (5.89)	-0.21 (11.3)
ECAS ALS-Specific	87.62 (5.86)	76.46 (14.18)	89.83 (6.7)	76.79 (14.72)	0.38 (5.5)	-1.71 (10.5)
Language	27.16 (1.01)	25.54 (2.77)	27.38 (1.17)	25.63 (2.8)	0.08 (0.88)	-0.26 (2.62)
Executive	41.22 (4.51)	35.54 (7.97)	42.21 (5.11)	35.58 (7.51)	0.58 (2.24)	-0.42 (4.58)
Social Cognition	11.87 (0.35)	11.15 (2.34)	11.75 (0.85)	11.37 (1.68)	-0.29 (5.21)	-1.03 (6.84)
Fluency	19.24 (1.96)	15.39 (5.62)	20.25 (2.23)	15.58 (6.41)	-0.17 (0.92)	-0.05 (1.92)
ALS-non-Specific	29.81 (3.37)	24.48 (7.25)	31.42 (2.77)	26.68 (5.84)	1.58 (3.49)	1.5 (4.59)
Memory	17.92 (3.39)	13.37 (6.14)	19.79 (2.64)	15.58 (4.95)	1.88 (3.64)	1.71 (4.22)
Visuospatial	11.89 (0.32)	11.12 (1.79)	11.63 (0.58)	11.11 (1.37)	-0.29 (0.62)	-0.21 (1.04)

^a CAG-repeat length was available for n= 41 PwHD at T1 and n=31 PwHD at T2.

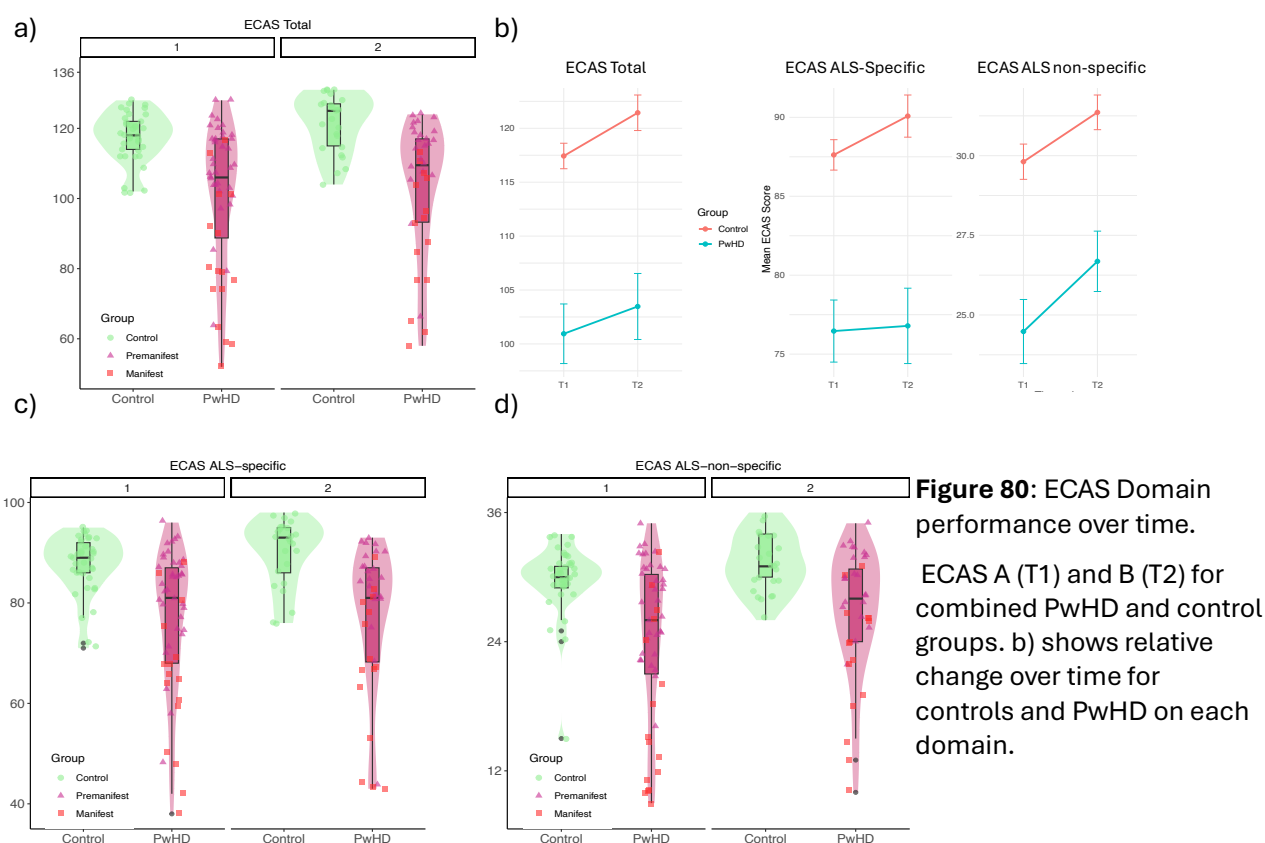


Table 67: Abnormality rates for PwHD based on published age- and education-matched cut-offs.

		Abnormal T1		Abnormal T2		T1-T2 Reliable Change Index (A-B)	
		PreHD	Manifest	PreHD	Manifest	Decline	Improve
ECAS Total	n	6	11	6	6	9 ^d	2 ^a
	%	15%	78.6%	20.7%	66.7%	21.1% (PwHD)	5.3% (PwHD)
ALS-Specific	n	5	11	5	6	10 ^e	3 ^b
	%	32%	78.6%	17.2%	66.7%	21.1% (PwHD)	7.9% (PwHD)
ALS-Non-Specific	n	10	9	7	7	4 ^f	8 ^c
	%	26.3%	64.3%	24.1%	77.8%	10.5% (PwHD)	18.4% (PwHD)

Grouping in table=diagnostic categories at baseline. n=6 premanifest T1 changed to manifest at T2. Descriptives of reliable change below (a-f) are groups at T2.

^a n=1 premanifest, n=1 manifest (T2) participants

^b n= ^a, plus n=1 additional premanifest participant

^c n=1 control, n=5 premanifest, n=2 manifest (T2) participants. All independent

^d n=1 control, 2 premanifest, 6 manifest (T2)

^e n=2 controls, 2 premanifest, 6 manifest (T2)

^f n=3 premanifest, 1 manifest (T2)

NOTE: No control participants were abnormal on ECAS Total. n=2/37 (5.4%) controls were abnormal on ALS-Specific and n=1 on ALS-non-specific at baseline (T1). n=1/24 controls (4.2%) fell in the abnormal range on ALS-non-specific T2.

10.4.2 *Longitudinal linear mixed effects models*

To examine whether the ECAS is sensitive to longitudinal change, LMMs were applied (see sample characteristics in **Table 59** & **Table 66**). **Appendix 24** shows the full model outcomes for each variable. Groups differed significantly on baseline TOPF-FSIQ ($p < .001$). Likelihood ratio indicated that TOPF-FSIQ improved model fit (ECAS Total: $\chi^2(1) = 11.8$, $p = .001$) and so was included as a covariate in all ECAS models. Although groups were matched for age, education, and interval between testing (median[IQR] = 20.3[11.7]), these variables can influence ECAS scores. Their inclusion was investigated using maximum likelihood estimation and ANOVA. Likelihood ratio tests indicated that the addition of age, education, sex and interval did not significantly improve model fit (ECAS Total: all $p > .64$) and so were not included as covariates:

Final model: ‘ECAS domain’ ~ TOPF FSIQ + group(T1) × timepoint + (1 | id)

Linear mixed effects models were constructed with ML estimation for each ECAS domain and subdomain that showed good sensitivity and convergent validity in the previous sections (all but visuospatial), allowing the intercept to vary by subject as the random effects (1 | id). There are high levels of heterogeneity across the ECAS models (Breusch-Pagan test: $p < .05$), likely due to score variation in manifest and premanifest groups, which violates the assumption. The assumption of normality of residuals was also violated in a minority of models, but to a small degree, which can be well handled by these models. Robust standard errors were computed using the ‘CR2’ sandwich estimator, clustering by participant ID, to correct for non-constant residual variance and mild non-normality. The ALS-non-specific and ECAS Memory models met all the assumptions, and so robust standard errors are not required.

Baseline TOPF-FSIQ was a significant independent predictor of ECAS Total, ALS-specific, Language, and Executive domain scores ($\beta = 0.27$ – 0.36 ; all $p_{\text{CR2}} \leq .001$), and showed trend-level associations with Fluency, Memory, and ALS non-specific domains ($\beta = 0.14$ – 0.16 ; $p/p_{\text{CR2}} = .077$ – $.094$). ECAS scores remained stable over time across most domains, with the exception of the Memory domain, which showed significant improvement between T1 and T2 ($\beta = 0.35$, $p = .018$). Timepoint effects were non-significant for other domains (β range = 0.05 – 0.26 ; all $p_{\text{CR2}} > .05$), though a trend was observed for ALS non-specific scores ($p = .057$). Manifest HD participants scored significantly lower than controls across all domains, even after controlling for premorbid IQ. Standardised group

effects were large ($\beta = -1.20$ to -1.91), reflecting substantial fixed effects of disease status on cognitive performance.

10.4.2.1 ECAS Total

For ECAS Total, three cases were deemed to have high leverage. All were manifest HD participants, two had particularly low scores, and one had high scores for the manifest HD group. Sensitivity analyses removed these cases and found the interaction of group (manifest) and time became significant ($B(SE) = -9.84(3.45)$, $p = .006$), in that the manifest group scored significantly lower across timepoints, scoring 9.84 points less on the ECAS at time 2. However, these scores were genuine and so cases were retained with robust standard errors are reported. The model did not find a significant interaction of group and time for manifest (robust SE applied: $B(SE) = -5.43(5.67)$, $\beta = -0.46$, $p_{CR2} = .352$) or premanifest PwHD groups (robust SE applied: $\beta = -0.09$, $p_{CR2} = .648$), controlling for TOPF-FSIQ scores. Manifest HD group x time interaction shows large standardised beta values for ECAS Total and ALS-Specific models (both $\beta = -0.46$), which approached significance for the uncorrected parameters.

10.4.2.2 ALS-specific domain

The ALS-specific model showed two manifest HD cases had high leverage (both low scores at T1). Removal of the cases in sensitivity analysis did not change the direction nor significance of the model outcomes. However, the p-value which approached significance in the initial model ($p = .068$) for the interaction of the manifest group and time now became significant (sensitivity analysis: $B(SE) = -7.25(3.23)$, $p = .028$). This suggests that the manifest group declined from T1 to T2 on the ALS-specific domain, holding premorbid IQ constant. As these scores were genuine, they were maintained in the model, meaning no significant interaction for group and time was detected after robust standard errors were applied (manifest*timepoint: $B(SE) = -6.01(5.59)$, $\beta = -0.46$, $p_{CR2} = .298$; premanifest*timepoint: $\beta = -0.09$, $p_{CR2} = .508$).

The largest effects were seen in the ECAS Fluency domain, where manifest participants significantly declined from T1 to T2 on the ECAS, with a large standardised beta coefficient ($\beta = -0.79$). This model found manifest HD participants declined 4.08 points on the Fluency domain, across the two timepoints (robust SE applied: $B(SE) = -4.08(1.89)$, $p_{CR2} = .047$). The Fluency domain showed excellent convergent validity, perfect sensitivity and excellent specificity on both ECAS forms (sections above), indicating that this domain may also

be the most sensitive to change over time on the ECAS for manifest HD participants. It did not show a decline at the premanifest group level ($p_{CR2}=.92$).

10.4.2.3 ALS non-specific domain

Trends of improvement over time were evident in the Memory and ALS-non-specific domains (ALS-non-specific: $B(SE)=1.57(0.81)$, $\beta=0.26$, $p=.057$), and the premanifest group trended lower than control participants across timepoints (ECAS Memory: $B(SE)=-1.88(1.11)$, $\beta=-0.35$, $p=.054$).

There were two influential points in these models however (one manifest and one control). Both scored low for their groups. Sensitivity analyses removed these cases and showed a significant group level difference for premanifest HD (ALS-non-specific: $B(SE)=-2.5(1.07)$, $\beta=-0.43$, $p=.021$).

10.4.3 Associations to CAP100 & disease progression

For the PwHD sample who had CAG data available, model comparisons using likelihood ratio tests indicated significant improvements in model fit at each step. Adding CAP100 to the baseline model significantly improved fit.

Final model for PwHD: ‘ECAS domain’ $\sim$ TOPF-FSIQ + timepoint + CAP100 + (1 | id)

10.4.3.1 ECAS Total

The final ECAS Total model explained both individual differences in intercepts (random effect; $SD=13.86$) and systematic fixed effects of estimated disease burden and premorbid cognitive ability. Higher CAP100 scores were associated with poorer ECAS Total performance, with a marginal non-significant effect ($B=-0.21$, $\beta=-0.3$, $p=.098$). TOPF-FSIQ showed a trend-level positive association with ECAS Total score ($B=0.48$, $\beta=0.30$, $p=.093$), so that ECAS Total increased 0.48 units for every unit increase in TOPF-FSIQ score. Although slightly increased, there was no significant change in ECAS Total scores between T1 and T2 ($B=0.44$, $p=.831$), indicating stable global cognitive performance over time in PwHD.

10.4.3.2 ALS-specific

The ECAS ALS-specific domain model for all PwHD with CAG data showed individual random effects variation for ID to be, $SD=8.68$. Higher TOPF-FSIQ scores were significantly associated with better ALS-specific cognitive performance ($B=0.42$, $\beta=0.37$,

$p=.031$), with ECAS ALS-specific scores increasing by 0.42 points for every unit increase in TOPF-FSIQ. CAP100 showed a non-significant trend, with higher scores predicting lower ECAS scores ($B = -0.13$, $\beta = -0.25$, $p=.133$). Although a negative value indicates a decrease in ALS-specific scores, there was no significant difference between T1 and T2 ($B = -2.16$, $\beta = -0.16$, $p=.266$), indicating stability.

10.4.3.2.1 ECAS Executive

Higher TOPF-FSIQ was significantly associated with better ECAS Executive domain scores ($\beta=0.37$, $p=.028$). CAP100 and timepoint were not significant predictors ($p>.19$). The marginal R^2 was 0.26, reflecting moderate explanatory power of fixed effects, while the conditional R^2 was 0.63, indicating substantial variance attributable to individual differences ($SD=4.74$).

10.4.3.2.2 ECAS Fluency

For ECAS Fluency domain, higher TOPF-FSIQ was marginally, but not significantly associated with better verbal fluency ($B=0.14$, $\beta=0.30$, $p=.084$). CAP100 and timepoint were not significant predictors ($p>.3$). A small, non-significant decline in fluency was observed over time ($B(SE) = -0.703(0.86)$, $\beta = -0.14$, $p=.42$). The marginal R^2 was 0.17, suggesting limited contribution of fixed effects.

10.4.3.3 ALS non-specific

ECAS non-specific scores significantly increased between T1 and T2 ($B=2.61$, $\beta =0.39$, $p=.003$), suggesting practice effects were evident on this domain. Higher CAP100 scores were associated with marginally poorer non-specific cognitive performance ($B = -0.08$, $\beta=-0.32$, $p=.098$), while TOPF-FSIQ was not a significant predictor ($B=0.05$, $\beta =0.09$, $p=.623$), indicating limited influence of premorbid IQ on ECAS domain scores in this model. Marginal R^2 was 0.16 and conditional R^2 was 0.8 (random effect; $SD=5.50$), indicating that individual differences accounted for most of the variance in scores.

10.5 Discussion

This chapter details the validation of two serial forms of the ECAS (A and B), which show excellent psychometrics in a sample of PwHD and controls. Overall, the main ECAS domain scores demonstrate excellent convergent validity, as did fluency and memory across both versions. The longitudinal study applied published RCI, identifying cases of meaningful decline over the study period. Group-level cognitive changes were less evident over time and in relation to CAP100-disease progression. Mirroring the strong diagnostic accuracy of the ECAS in ALS^{162,163}, this study substantiates the ECAS as a viable cognitive screening tool for HD.

10.5.1 *ECAS sensitivity & specificity analyses*

10.5.1.1 *ECAS A, T1*

ECAS A Total score had very high convergent validity with the gold-standard battery, as did the ALS-specific and Fluency domains. The Total score and ALS-Specific composite showed optimal sensitivity and specificity profiles, reinforcing the utility of the ECAS for identifying fronto-striatal dysfunction in HD and distinguishing cognitively intact individuals with high confidence (NPV=0.986 and 1.0 respectively). Group-level impairment rates in the Executive domain are well matched between ECAS and extended assessment tasks in manHD: 35.7% on both although, the ECAS slightly overcalls impairment relative to the extended battery (e.g.: 7.9% vs 2.6% in premanifest). But as a screening tool, this is preferable to false negatives.

Low PPV on many of the task domains implies that the ECAS may be oversensitive to impairment (false positives), possibly due to its briefer format or lower specificity of tasks. This is particularly evident on the Executive domain. Although group-level rates of Executive impairment were similar between the ECAS and extended neuropsychological battery (35.7% in manifest patients), individual-level classification agreement was low. ECAS executive function showed particularly low sensitivity (0.33) and PPV (0.125), likely reflecting a floor effect or insufficient task complexity within the ECAS subtests. Visuospatial performance remained the least sensitive, identifying less than one-quarter of impaired individuals, but had perfect specificity and PPV suggest it is only detecting the most severe cases. This may be expected given the limited scope of ECAS tasks targeting this domain.⁶³⁵

Looking at individual subtests, the DSB and Alternation combination, and sentence completion and CWIT Inhibition time in the ECAS executive domain do not

correlate as highly as the other tests. While in principle these tasks tap similar executive constructs, the CWIT Inhibition, as discussed in **Chapters 7 and 8** is largely confounded by processing speed, which may not necessarily correlate with sentence completion on the ECAS, which is not timed. The alternation task is also not forgiving to early mistakes, which the DSB would allow recovery for. In any case, the original validation of the ECAS cautions against interpretation of particular tasks, instead favouring the main domains to inform follow-up decisions.^{163,777}

For screening tasks, a balance diagnostic validity, including high sensitivity, specificity, and positive and negative predictive value (PPV/NPV) is desirable. Higher sensitivity and NPVs are prioritised as it minimises the risk of missed cases. False positives can be resolved through more comprehensive neuropsychological follow-up, which should follow an abnormal screening outcome.

While the ECAS shows slightly less sensitivity in HD than in the original validation for ALS, it shows improved specificity and PPV in main domains of interest (Total, ALS-Specific, Non-Specific) and appears to demonstrate improved all round performance in the memory domain.¹⁶³ This is likely due to the magnitude of impairment evident on memory tasks in HD relative to ALS, where memory is generally spared. Overall, the ECAS performs well under a conservative impairment criterion, particularly for screening out unimpaired individuals with high confidence (NPV).

Using an Italian version of the ECAS, Carelli et al.⁷⁷⁶ found PwHD were globally impaired, scoring lower than PD and ALS groups across all domains. The current study showed higher sensitivity and specificity of the ECAS than this study, likely due to the use of the full gold-standard battery.

10.5.1.2 ECAS B, T2

The ECAS version B at T2 demonstrated strong convergent validity with standard neuropsychological tests, particularly for verbal fluency, memory and the digit span. Composite correlations were also robust, with ECAS ALS-Specific score and Total score showing the strongest alignment. Sensitivity and specificity metrics were broadly consistent with previous ECAS validation work^{163,778} with all ALS-specific domain scores showing sensitivity/specificity above 0.8/0.87. The ECAS B Total score showed high specificity (0.873) and excellent negative predictive value (NPV=0.96), confirming its reliability in ruling out impairment. However the sensitivity of the Total score was likely impacted by the very low visuospatial sensitivity (0.143). High specificity (0.956) for the visuospatial domain, suggests poor detection of impairment unless deficits were severe.

This echoes findings that visuospatial subtests in the ECAS fail to capture subtle deficits, possibly due to task simplicity or ceiling effects.^{635,776}

Fluency and memory domains achieved perfect sensitivity with excellent NPVs. Language and executive scores also performed well, though positive predictive values (PPV) were moderate, indicating some risk of false positives for these domains.

Compared to ECAS A, version B showed modest improvements in memory, executive, and language sensitivity. High proportions of abnormal scores were captured across domains in manHD, and for premanifest participants.

10.5.2 *The ECAS executive domain*

The ECAS executive domain performed below expectations on sensitivity and specificity analyses using published cut-offs. ROC curves and resultant revised cut-offs of 31.5 for abnormality yielded improved classification accuracy (AUC = 0.875). Similar analysis for the primary domains (Total, ALS-specific, ALS-non-specific) did not improve upon the already excellent classification of these scores for ECAS A. This may support the case for HD-specific thresholds for the executive domain.

Although both are significant, the ECAS social cognition subtest as well as the visuospatial domain show limited convergent validity for ECAS B ($r < 0.31$). Although the executive domain which it contributes to was much improved in ECAS B, its PPV remains low. Consistent with previous criticisms that it predominantly assesses visuospatial gaze processing, the social cognition task may not tap the same broader ToM or social reasoning that the RMET demands.⁷⁷⁸ As highlighted above, the CWIT Inhibition may not adequately reflect the constructs being assessed by the ECAS sentence completion task in this pairing, as their convergent validity was lower than other subtests on ECAS B also.

Tjokrowijoto et al. (2023)⁷⁷⁸ recently highlighted the limitations of the ECAS executive function domain in general, which show limited sensitivity to inhibition as well as to social cognition tasks. This may also explain poor performance of the executive domain on ECAS A, discussed above.

Finally, ECAS domains, particularly memory and language, tended to over-identify impairment compared to gold-standard tests. While this enhances sensitivity, it reinforces the ECAS's role as a conservative screening tool rather than a diagnostic instrument. Abnormal classification should be followed by in-depth neuropsychological assessment to confirm cognitive status.

10.5.3 *Longitudinal ECAS performance*

LMMs constructed for group at baseline with the interaction to time found that manifest HD participants scored significantly lower than premanifest and control participants on all ECAS domains, controlling for TOPF-FSIQ, concordant with findings reported in the last chapters on the extended HD-Cognition battery. There was high heterogeneity in the models due to variability in cognitive function of PwHD signalled by heteroscedasticity of the residuals, high conditional R^2 and random intercept variance. The only significant interaction of group and time was for the manifest HD participants on the ECAS Fluency domain, indicating scores for manifest participants declined from T1 (ECAS A) to T2 (ECAS B) where others mostly improved over time. This is pertinent, as the ECAS Fluency domain controls for motor dysfunction, and reduces the impact of more general speed of processing deficits. Given the Fluency domain showed the highest sensitivity, specificity and NPV of all ECAS domains, and was not associated with premorbid IQ, it suggests that, it is the most sensitive to progressive cognitive impairment in this relatively small manHD sample.

Looking to rates of impairment and reliable change, the ECAS captured a high proportion of abnormal scores for manHD, but also in preHD. Combining the groups, the RCI identified 21% of PwHD experienced meaningful decline from T1 to T2 on ALS-specific and Total domains, compared to 10.5% on non-specific. However the number of decliners is small, and a relatively high proportion significantly improve by the RCI, 18.4% in the non-specific domain.

10.5.3.1 ECAS scores & disease factors over time

The final analysis evaluated the influence of CAP100-estimated disease progression on ECAS performance over the two timepoints. Not reaching significance in any model, CAP100 was a modest but consistent predictor of poorer performance on the ECAS, most notably for the Total and ALS non-specific domains, where associations approached significance. However, the effects were marginal, suggesting that CAP100 score may have limited predictive utility for short-term cognitive change using the ECAS, particularly in presymptomatic stages. Conversely, the ALS non-specific domain improved between T1 and T2 both in the overall group model and CAP100-disease progression model, but was the only domain to demonstrate practice effects. Important to consider in longitudinal cognitive assessment, the RCI accounts for practice when determining reliable change over time.

Premorbid cognitive ability (TOPF-FSIQ) was a stronger and more reliable predictor of cognitive performance than CAP100, especially in the ECAS Total, ALS-Specific, and Executive domains. Fluency and ALS-non-specific tasks approached significance, but were less impacted by premorbid function. A substantial amount of the variance in ECAS score is explained by education, and further still by IQ (approx. 24%).⁷⁷⁹ Although this is not as high as other screening tools, it reinforces the need for demographically-matched normative cut-offs and longitudinal assessment, especially in neurodegenerative disease where deviations from an individual's baseline are often more clinically informative than cross-sectional scores alone.

10.5.4 *Study-specific limitations*

The ECAS was administered alongside the extended neuropsychological battery in HD-Cognition. Performance is not therefore in isolation as a cognitive screening tool would ordinarily be used. The order of administration was randomised across participants to control for order effects, maximising generalisability.

In model selection, the TOPF was found to improve fit above age and education, which are usually used to demographically control for their effects on ECAS cognitive outcomes. Using these as predictors may have changed the results. However, the TOPF-FSIQ is computed based on a participant's age, education, and sex, and therefore along with premorbid IQ estimations, likely offers more control for demographic group differences.

Across both versions of the ECAS and in all groups, rates of impairment are much higher in the RCFT – copy trial than the ECAS visuospatial domain. This may be as the normative scores penalise harshly any dropped points on the copy trial, whereby getting 32 points out of 36 would give someone with high education in their 50s a z-score of -2.5. This may be overly sensitive to actual impairment in visuospatial and visuoconstruction abilities, and is also inhibited by motor dysfunction (drawing the figure with pencil and paper), where the ECAS subtests are not (performed by visual inspection).

10.6 Conclusions

These findings support the ECAS as a quick and valid cognitive screening tool in HD, consistent with its utility demonstrated in ALS^{159,163} and improving upon recent studies in HD and PD.⁷⁷⁶ With good sensitivity and specificity for ECAS Total on both A and B, and excellent negative predictive value (NPV>0.95), it reliably rules out impairment. However, similar to the original validations in ALS, despite core domains demonstrating high sensitivity and NPV, the ECAS is limited by poor PPV. Therefore interpretation of individual subtests is cautioned.

ECAS A and B also proved sensitive to longitudinal cognitive decline over a median follow up of 20 months. It did not detect cognitive decline at the group level for preHD, but RCI cut-offs capture a number of premanifest and manifest PwHD who decline significantly over the study period, further supporting its utility as a clinical cognitive screening tool in HD. Overall the Fluency domain emerges as a promising marker of cognitive decline in manifest HD with excellent psychometrics all round. The widespread adoption of the ECAS in ALS clinics, clinical trials and research, the now widespread availability of alternative languages, local normative data and clinical cut-offs make it an attractive candidate for use in HD also.

11 Chapter 11:

Discussion, Limitations, Conclusions and Future Directions

11.1 Introduction

The preceding study chapters apply a neuropsychological phenotyping approach to ALS and HD, highlighting the diversity of extramotor presentations in neurodegenerative disorders of the motor system. The primary objective of this thesis was to investigate the cognitive and behavioural profiles associated with ALS and HD, with a particular focus on apathy, verbal fluency and social cognition in early-stage and at-risk individuals as transdiagnostic markers of network dysfunction. To achieve this, eight overlapping aims were specified: Aim 1 was to validate the online neuropsychological assessment battery against standard in-person administration. Aim 2 evaluated the influence of the clinic environment and recent diagnosis on ECAS performance in pwALS. Aim 3 examined the profiles and cognitive correlates of multidimensional apathy across HD and ALS. Aim 4 assessed cognitive endophenotypes in unaffected first- and second-degree relatives of familial ALS patients. Aim 5 investigated social cognitive dysfunction as an early marker of HD, focusing on premanifest stages. Aim 6 explored contextual and individual factors affecting neuropsychological performance, including IQ, apathy, fatigue, and motor function. Aim 7 introduced and tested a new index of relative phonemic fluency (RFI) as a potential cognitive marker of ALSci. Aim 8 evaluated the sensitivity and specificity of the ECAS for cognitive screening in HD.

These aims and their associated hypotheses arose throughout this thesis, not necessarily in order, and sometimes in multiple studies and chapters. This final chapter synthesises and highlights the main findings in relation to these aims, situating them within the existing literature. Limitations of the study are discussed, and directions for future research are proposed.

11.2 Addressing study aims & hypotheses

11.2.1 *Aim 1: Online neuropsychological assessment*

Online assessment proved to be a reliable and acceptable medium for neuropsychological testing. The online battery demonstrated consistency to the gold-standard assessment in the online validation study (**Chapter 5**), confirming **Hypothesis 1.1**. Although it was a necessary methodological change to allow continuation of these studies during the Covid-19 pandemic, the format was well received by participants. It joins a growing literature base of tele-neuropsychology demonstrating reliability for these methods.^{593,627}

The use of Qualtrics for questionnaire data collection improved integrity and usability of behavioural/psychiatric data. The forced-response feature enhanced dataset completeness by making sure participants did not miss important responses. Though it may not replace in-person assessment, it is a viable alternative for those wishing to avoid the barriers associated with in-person testing.

11.2.2 *Aim 2: Distress & factors affecting cognitive performance*

11.2.2.1 *Cognition in the ALS clinic environment*

The second part of Chapter 5 found a large proportion (55%) of pwALS experience increased levels of subjective distress on the Distress Thermometer at their baseline visit to the MND Clinic. Despite this, distress was not associated with decreased cognitive screening outcomes, measured by the ECAS. None of the variables measured were significantly associated with subjective distress, with the exception of carer-rated apathy (DAS). Distressed pwALS were 12 times more likely to be rated as clinically apathetic by a caregiver.

Hypotheses 2.2–2.4 were not supported under this aim. There appears to be highly individualised contributors to distress in the context of an ALS diagnosis, which is supported by systematic review of the literature.^{648,656} This peri-diagnostic period confers increased risk for mood disturbance in pwALS¹⁹², which may have a longer prodromal phase.¹⁵⁷ A recent qualitative study has shown that pwALS experience distress throughout the disease course⁷⁸⁰, and so a quick screening tool for distress would facilitate monitoring. Based on the literature^{639,654}, a cut off of ≥ 6 may be optimal to economically and equitably assign services to those most in need. However, full validation and

sensitivity and specificity analyses will be needed to determine the most appropriate cut-off for patients with MND.

Cognitive and behavioural changes are present in a subset of pwALS, but contrary to **Hypothesis 2.3**, no significant associations were observed here. Importantly, while anosognosia is often linked to behavioural impairment, the two are not synonymous^{781,782} and it is less common in mild extramotor impairments in ALS.^{783,784} In any case, the strength of the DT is that it captures the patient's subjective experience.

Although travel distance was not a significant predictor of distress on the DT nor of ECAS scores in the ALS-patient study (**Chapter 5**) it has been associated with burden and worse health outcomes.⁷⁸⁵ Telemedicine and teleneuropsychology can be employed to combat this, increasing cost-effectiveness and efficiency⁶²⁸, and permitting access to services for patients in remote areas.⁶³⁴ The remote version of the ECAS is well placed to meet this need, and together with the findings of the online validation study support the medium generally.⁵⁹⁵

11.2.2.2 The ECAS

Mild-to-moderate psychological distress can affect performance on low-load cognitive tasks, where distraction may occur, but not for tasks demanding higher executive and attentional resources.⁶⁴⁹ Although other studies have shown some association to executive scores⁶⁴⁶, the ECAS is robust to subjective distress levels and the clinic-associated factors measured in this study. Highlighted by the audit in this chapter is the importance of the RCI for evaluation of classification and clinically meaningful declines on repeat assessment, as opposed to cross-sectional abnormality cut-offs, especially for those with lower education, older age and who may have a lower premorbid cognitive ability.

Distress, apathy and cognitive symptoms have direct implications for daily functioning and quality of life in ALS and HD. These deficits affect motivation, interpersonal relationships, and the ability to perform activities of daily living.^{358,387,786} Findings from this thesis support the use of cognitive and behavioural screening tools like the DT, ECAS and DAS to identify individuals at risk of poorer functional outcomes in both ALS and HD. Embedding such assessments into clinical pathways in HD as they are in ALS may enable timely psychosocial support, tailored interventions, and improved allocation of resources.

11.2.3 *Aim 6: Other factors affecting cognitive assessment*

A theme throughout this thesis is the acknowledgement of the task impurity problem^{523,524}, and an attempt to mitigate the effects of multiple competing constructs for, a) a more reliable measure and b) a more valid interpretation of the neuropsychological construct in HD in particular. Aim 6 was to quantify these effects across disorders, tests and studies, acknowledging concurrently the limitations and pragmatism of cognitive assessment with a view to improve reliability and validity of interpretation. Aim 6 addressed hypotheses within **Chapters 7, 8 and 9**.

Verbal IQ was shown to be associated with performance on the RMET here, underscoring the need for carefully matched control groups in evaluating cognitive function. Contrary to the **Hypothesis 6.1**, and studies citing overreliance on language^{679,685}, there was no evidence of a threshold effect. Verbal IQ (WASI-II Vocabulary) was also associated with ACS-SP Affect Recognition, indicating that the influence of verbal ability is no more impactful to the RMET than tasks with high-frequency common words, when controlling for premorbid ability.

11.2.3.1 *Fatigue & cognition*

Discussed at the end of the cross-sectional HD-Cognition analysis and the chapter on apathy, fatigue is prevalent across HD disease stages, including preHD. Mental and Emotional fatigue were particularly high in the current study. Similar to the findings on the effects of tiredness on the ECAS (**Chapter 5**), and in support of **Hypothesis 3.4**, pervasive fatigue demonstrated a negative association to cognitive scores, but did not account for group differences in cognition in the models employed. Studies in MS report fatigue may increase subjective cognitive complaints, though its effect on objective cognition is less established, in line with the current findings.^{705,706} Given the reduction to quality of life associated with increased fatigue in this study, and in the literature, it points to fatigue as a modifiable target for improving QoL and cognitive function^{786,787}, though the effect on the latter may be modest.

11.2.3.2 *Controlling for motor impairment & processing speed*

The control of motor dysfunction in evaluation of cognition and behaviour in ALS is well established, with tools such as the Verbal Fluency Index (VFI)^{150,788}, DAS⁴³² and the BBI.^{153,578} Although researchers in ALS have attempted to apply these methods in HD⁷⁷⁶, they are less recognised in HD research. Comparisons of VFI and standard age- and

education-stratified normative data are made to assess for improvement due to control of motor function. It did not change the strength of the effects for fluency in manHD, but the VFI for semantic and phonemic fluency improved in preHD. Whilst motor dysfunction is not expected in clinically asymptomatic premanifest participants, this may reflect control exerted by the VFI for the variability due to speed in general in this sample, not just motor speed. This is supported by past work showing fluency tasks are better explained by speed than executive or linguistic constructs.⁶⁹³ The VFI may prove useful therefore to isolate higher order executive function in PwHD. Dysarthria and bulbar dysfunction in general are usually affected in late-stage HD²²⁴, and so the effects in this early-manifest sample may not be apparent. Although not definitive, findings are suggestive of **Hypothesis 6.3**. Future work should assess the VFI for use in a later-stage HD sample.

11.2.3.2.1 Speed & executive function

Tasks assessing executive function often do not control for the effects of processing speed, leading to an overestimation of executive dysfunction in HD populations. Chapter 7 and 8 demonstrate significant attenuation of effect sizes when controlling for task-based speed factors on the CWIT Inhibition subtest in PwHD, as anticipated in **Hypothesis 6.3**. Error scores and non-timed executive scores, such as the DSB are still reduced however, even in preHD and with preserved simple attention on the forwards condition. This demonstrates genuine executive difficulties for working memory and task monitoring in HD, but to a much lesser degree than speeded tasks. Evidence from the literature for discrete executive deficits in working memory, set-shifting and error monitoring for example, correspond to putamen and striatal neuroimaging markers.^{491,789,790}

Fronto-parietal cortex and striatal atrophy underlie processing speed deficits in HD^{255,702}, whereas fronto-striatal network dysfunction cause executive deficits.^{251,501} Network abnormalities affect dorsolateral-striatal circuit function (task setting and monitoring)³³², evidenced here by significantly reduced processing speed effects, with only minor effects to error monitoring in manHD, and no effects in preHD. Together this suggests that that parietal and striatal degeneration may exert a stronger influence on cognitive outcomes than more distributed network-level dysfunction.

These results emphasise a need for caution when interpreting neuropsychological tests in neurodegenerative populations. The CWIT, SDMT and the Trail Making Test, for example, are good biomarkers for early cognitive dysfunction in

HD^{255,262,341,392} and FTD⁵¹¹, but do not demonstrate validity when impairment is attributed to inhibition, switching, flexibility and other higher order latent executive constructs without control for reduced speed of processing.

11.2.3.2.2 Behavioural measures in motor disorders

An unexpected finding in the apathy chapter, was the association between ALSFRS-R scores and the DAS Initiation apathy domain in pwALS, previously unreported.¹⁵⁴ Whilst this may be evidence of shared disease progression, supported by an association to behavioural impairment on the BBI and ECAS Fluency, it may also reflect the confounding effects of deteriorating motor function. Self-report tools can exaggerate what many researchers and clinicians accept as primary behavioural changes, without acknowledgement of the source. Despite endeavouring to minimise the effects DAS, Initiation apathy questions are highly related to physical movement and motor disability. Anecdotally, when outcomes are clarified, respondents often report changes being associated with patient motor disability. However, these findings should be examined further to validate symptom presentation in pwALS in the real-world clinic setting.

11.2.4 Aim 4: Endophenotypic traits in ALS

Findings from **Chapter 6** furthers previous work from our group which show unaffected fALS relatives display subtly reduced cognitive performance on a range of cognitive measures.^{194,210,211}

11.2.4.1 Replication of the cognitive endophenotype

Random-effects meta-analysis and z-tests provided moderate support to the hypothesised replication of putative cognitive endophenotypes in Phase 1 of the MND Family study (**Hypothesis 4.1**). Of the primary tests of interest based on Phase 1, confrontation naming and the RFI met all criteria for outright replication, demonstrating equivalent effects in both phases. Working memory trait differences also evidenced replication. DSB effect sizes were smaller and non-significant in the smaller replication sample alone, but consistent between phases and of medium effect in the pooled analysis ($d=.56$).

The large Phase 1 effect for the FAS task was not evident in the Phase 2 replication. Nevertheless, the RFI did differentiate the groups, with a medium effect size ($d=0.49$), demonstrating low heterogeneity, and consistent direction and magnitude of effects, therefore supporting evidence of differential fluency performance and replication in both

phases. Additional support for the presence of discrepant fluency performance comes from the longitudinal Phase 2 study, also addressed in this chapter. fALS relatives in the longitudinal study scored consistently lower than controls on the FAS task across time. The effects did not reach significance when controlling for premorbid IQ estimates however.

These core domains typify ALS-specific cognitive impairment in the disease phenotype, providing strong support for their continued investigation as putative cognitive endophenotypes.^{16,118,141,163,532} However, as FAS task performance failed to replicate, and as the RFI is a novel measure, the observed fluency effects should be interpreted with caution, pending further validation of the RFI.

11.2.4.2 Endophenotype temporal stability

Assessment of trait differences in a longitudinal design was also an objective of the MND Family study. **Hypothesis 4.2** found tentative support for temporal stability of the candidate cognitive endophenotypic traits. As with the replication study, confrontation naming exhibited the strongest evidence for stability by the two methods used. In the case of all six key variables, fALS relatives scored lower than controls across time, and did not decline, showing equivalent practice effects. Despite demonstrating stability and substantiating the hypothesis, when controlling for the effects of premorbid intellectual ability, group differences were only significant for the BNT after correction for multiple comparisons. This is likely due to the smaller sample size in the Phase 2 longitudinal study, and additional controls for premorbid IQ endeavouring to address demographic differences.

11.2.4.3 Implications of endophenotypic traits

Endophenotyping complex genetic disorders (such as ALS) promotes a bottom-up understanding of aetiology by indexing genetic vulnerability. It can have transdiagnostic benefits to understanding the causative mechanisms leading to the emergence and trajectory of clinical symptoms that are more valid and pharmacologically targetable, complimenting existing eponymous classifications.⁷⁹¹ By their nature, the magnitude of endophenotypic traits are small. They are not likely to convey an individual risk profile, but demonstrate power at the group level, in sample sizes typically associated with explorative genomic analyses.

Presence of endophenotypic traits in unaffected fALS relatives observed here, suggests that unknown genetic mechanisms are mediating trait expression. The

subclinical cognitive and trait differences described in relatives were driven mostly by those with a positive family history of ALS, as opposed to the presence of the *C9orf72* mutation.^{194,210} This suggests that unknown genetic mechanisms could be mediating the putative cognitive endophenotype observed here, in the fALS relative cohort especially. It presents an opportunity to explore a new area in the genetics of ALS as it relates to cognition.

Identifying endophenotypes in ALS holds significant potential for advancing gene discovery and clarifying oligogenic disease mechanisms. This approach has been effective in other conditions, including schizophrenia, where novel genetic loci associated with cognitive traits have been discovered which contribute to disease risk.²⁰⁵⁻²⁰⁷ Current findings add necessary steps to the initial foundations laid by Costello and Ryan, and are significant steps towards validating these cognitive endophenotypes of ALS, which ultimately could enhance the gene discovery process. It did not however allow differentiation of *C9orf72*-associated neurodevelopmental effects and endophenotypes. However, as C9+ participants did not perform differently from C9- participants, even over time, alterations in cognitive performance cannot be attributed to *C9orf72* in these unaffected individuals.

Beyond gene identification, these putative endophenotypes may also support improved diagnostic precision of pwALS through a similar methodology to the Research Domain Criteria.^{399,403,404} This could inform sample selection and enrichment for clinical trials, facilitate early intervention, and the development of clinical pathways for at-risk individuals.

11.2.5 *Aim 4: Cognition in unaffected carriers of C9orf72:* *presymptomatic diseases vs asymptomatic trait*

In both cases of findings from the MND Family study discussed above, the effects of trait differences were present independently from *C9orf72* status, i.e., C9+ and C9- at-risk relatives did not differ in their performance. Sub-studies of unaffected at-risk relatives and pwALS in **Chapter 6** and **Chapter 9** explored the effects of *C9orf72*-carriership directly.

11.2.5.1 Asymptomatic C9orf72-carriers

In fALS relatives, there were no significant differences between genotypes across the extended neuropsychological test battery or the ECAS in Phase 1 or the Phase 2

longitudinal study. For the most part, increased scores were evident in both groups between timepoints, with no group-level decline, consistent with other studies.^{100,186} Reliable change index scores indicating decline on ALS-specific domains for two middle-aged *C9orf72* carriers is tentative evidence for **Hypothesis 4.4** and somewhat concordant with other longitudinal studies in C9-ALS patients.¹⁷³ Extended battery neuropsychological test data were not available for these cases. Whilst the ECAS and RCI may demonstrate utility as early cognitive markers in asymptomatic *C9orf72* carriers, findings should be interpreted with caution given the small number who declined.

Crucially, the current studies compared at-risk relatives to each other. Other studies have demonstrated neuropsychological differences in *C9orf72*-carriers when compared to healthy controls, most notably on phonemic verbal fluency.⁴⁸⁹ C9- and C9+ at-risk relatives did not appear to differ from each other in this study however, suggesting that gene status alone may not fully account for cognitive differences. Presentation may instead reflect broader familial or kindred-related influences, more in line with the shared disease liability as endophenotypes, hypothesised to occur here. Some minor signals were evident in the longitudinal design of the MND Family study, presented in **Chapter 6**, but which could not be further explored in the Phase 2 replication cohort due to the unavailability of genotype data. The current studies do not have sample sizes which would conclusively address the hypotheses relating to C9-carriership.

11.2.5.2 Symptomatic *C9orf72*-positive pwALS

In the large clinic-based sample of pwALS in **Chapter 9**, the only difference by genotype was a one point median difference in the C9-positive pwALS on the ECAS Visuospatial domain. No other variable differentiated the groups. Although *C9orf72* is associated with more a severe phenotype and cognitive/behavioural symptoms⁶⁸, this finding of no effect is not uncommon in the literature.^{173,753} This has implications for ‘presymptomatic’ studies, if symptomatic C9-ALS patients do not show the characteristics being probed for in unaffected populations.

11.2.5.3 Investigating “presymptomatic” change

Another factor to consider in presymptomatic studies is the testing interval. The HD-Cognition study spanned 1.5-3 years for 2-3 timepoints. This is a relatively short observation period compared to the typical 20-year disease course of HD, especially when the primary focus was on the premanifest phase, where change is slow. The longitudinal aspect of the MND Family Study, although spanning a considerable time-

frame (up to 6 years), may not have been sufficient to detect longitudinal decreases in performance either, should prodromal disease-factors have presented. Decline in cognitive function in symptomatic C9-ALS patients is not reliably detected over 12 to 18 month study periods^{100,115}, and other studies of asymptomatic C9-carriers have not shown deterioration over 12 month periods.⁴⁸⁹ This remains a challenge in presymptomatic research, where unpredictable disease-onset limits the ability to enrich samples and thus detect change over study periods. TOST analyses were used to maximise the validity of absent effects in the current studies (**Chapter 6**).

In addition, HD-Cognition focuses on premanifest and early-stage HD. PwHD had relatively low CAG-repeat lengths (~42), indicating a higher predicted age-of-onset and slower disease progression.³¹⁴ Manifest participants were typically older with high, stable functional scores. Similarly, this cohort of asymptomatic C9-carriers is relatively young. These samples align with intended study populations, but slow progression limits detectable change in both studies over the study period. Longitudinal studies are essential for the investigation of evolving phenotypes from the presymptomatic phase and so these studies provide excellent opportunities for continued longitudinal investigation of cognitive traits, associated with the endophenotype, as well as the effects of *C9orf72*-carriership.

11.2.6 *Aim 3: Profile of apathy in ALS & HD*

Consistent with the literature and theoretical models, rates of clinically significant apathy were between 36.8% and 62.5% across conditions.^{154,381,384,441} The profiles diverged in ALS and HD, with Initiation apathy common to both. Executive apathy was also significant in the PwHD profile, providing support for **Hypothesis 3.1** and in agreement with Atkins et al.⁴⁴¹ In a factor analysis of the Lille Apathy Rating scale, the arising dimensional structure mapped on well to the DAS, and showed specific patterns of atrophy related to the components of apathy.⁷⁹²

Over ¼ of premanifest participants endorsed clinically significant apathy on at least one domain. Not previously reported using the dimensional framework, the profile of apathy in preHD also matched that of manHD, with milder severity. Clinically abnormal behavioural presentations in asymptomatic individuals by motor-diagnostic criteria underscores the need for extramotor clinical diagnostic criteria.^{237,307} Such criteria would

improve sensitivity to the heterogeneous clinical presentation of HD³⁰¹, and allow earlier access to services for those affected.

11.2.6.1 Apathy & depression

Rates of depression were expectedly high in this sample; 56% and 23% moderate-severe in manHD and preHD respectively, roughly equal to apathy. Depression did not account for apathy in the manHD group, but was highly related to, and accounted for apathy scores in the premanifest group. Thus **Hypothesis 3.2** received partial support. In accordance with findings that depression is not necessarily related to motor symptom onset^{424,747}, and the absence of association to disease progression estimates and functional measures used here (CAP100 and UHDRS), it suggests that apathy may be associated with psychological factors in earlier stages, primarily driven by symptoms of depression, but that apathy may dominate and progress through later stages.³⁸⁶ Although depression accounted for apathy at the group level in preHD, the characteristic apathy profile, shown in manHD (elevated Initiation and Executive apathy), was present to a lesser degree in a subgroup of premanifest participants. This profile did not change over time in the longitudinal design. Longer timeframes and larger samples of manHD may be needed to detect temporal dynamics in apathy. Further distinguishing their relationship with the HD extramotor phenotype, Baudic et al.⁷⁹³ found that PwHD with and without apathy differed on tests of executive function, attention and memory, where depression had no such effects.

11.2.6.2 Apathy & cognition

Theoretical underpinnings forecast increased Initiation apathy in pwALS, due to shared networks underlying ‘energisation’ and response-generation cognitive tasks which selectively impact phonemic verbal fluency, and in addition, Executive apathy and executive function in PwHD.³³² Across both cohorts, cognitive performance was associated with specific apathy domains, supporting the hypothesis that apathy subtypes map onto distinct cognitive profiles and fronto-subcortical circuits, although not for Emotional apathy, which was very low in this sample. This partially replicates the findings of Radakovic et al. (2017) in a large incident, clinic-based ALS sample (**Hypothesis 3.3**). However, broader overlap was apparent than was hypothesised, as executive pairings had not been reported before. Prognostically, apathy is associated with a more rapid general cognitive and functional decline over 2 years.³⁸⁷ These associations were not explored in the longitudinal design.

Hypothesis 6.2 was proposed to investigate the extent to which shared network abnormalities manifesting as dimensions of apathy and cognitive dysfunction, and the converse, the implications of reduced motivation and effort on cognitive test performance. This question is pertinent in light of recent findings that an increased sensitivity to the cost of effort (rather decreased reward responsiveness) underlies the symptoms of apathy in HD.^{434,439,440} While an association was demonstrated here, controlling for alternative factors, causality and the direction of this affect cannot be determined. Nonetheless, cognitive and apathy outcomes were associated on the extended battery in PwHD (Executive and Initiation; **Hypothesis 3.3**), but not for social cognitive function. The orbitofrontal-striatal network signal was lower on the DAS, despite affect naming/affective ToM deficits. This could be explained by floor effects and tool insensitivity for the DAS Emotional domain, and separately above for the ECAS Social cognitive domain, which have received criticism for sensitivity.

Studies in ALS have reported no significant associations between DAS apathy domains and the ALSFRS-R, counter to the findings here.⁷³³ However, a recent publication⁷⁴³ describe an ‘Initiation apathy’ only subgroup of pwALS, who are cognitively normal but more significantly motor-impaired, although not explicitly acknowledged. Associations could indicate that Initiation apathy increases with motor impairment, consistent with global disease progression selectively impacting the “energisation” construct underpinned by decreased salience-related processing in the ACC.^{332,442} Supporting this is the association with general behavioural impairment which was also demonstrated in the multivariate models, and separately, the association to ECAS fluency scores controlled for by the VFI, highlighting the shared cognitive/behavioural architecture.³³² However, it may also reflect bias, with scores confounded by deteriorating motor function. Strong and independent associations here raise concerns about the validity of DAS Initiation subscale that warrant further investigation.

Largely consistent with the HD findings here, and supporting the transdiagnostic utility of apathy measures for mapping neurocognitive mechanisms, Eggins et al.⁷³⁹ found that the DAS Executive apathy domain was significantly associated with impaired cognitive composites in both bvFTD and AD, showing differential impairment profiles independent of general cognition and disease stage.

The variability in the profile of apathy suggests multiple pathways contributing to its different behavioural and cognitive expressions. Depression plays a role in early disease, but the findings bear similarity to the organisation of the cortico-subcortical

basal ganglia functional networks highlighted in Parkinson's disease by Rodriguez-Oroz et al.⁷⁹⁶ and Jahanshahi et al.³²³ who cite distinct sensorimotor, associative/cognitive and limbic loops as governing three categories of facilitation and inhibition.

The assessment of apathy and its cognitive correlates in HD and ALS contributes insight into mechanistic associations, where cognitive impairment and apathy can, but do not always, co-occur. These findings underscore dimensional apathy profiles as clinically meaningful, transdiagnostic markers of fronto-striatal dysfunction which could be considered under the RDoC across conditions.⁴²⁵ Apathy has been the focus of recent transdiagnostic therapeutic trials, with groups evaluating treatments in HD⁴⁰¹ and FTD.⁷⁹⁷⁻⁷⁹⁹ Both a potential target for early intervention and a prognostic indicator for cognitive and functional decline, effective treatment of apathy could therefore modify disease trajectories and improve QoL across conditions, including HD and ALSFTSD.

11.2.7 *Aim 5: Social cognition & executive function in HD*

Chapters 7 and **8** show analysis of the baseline and longitudinal aspects of the HD-Cognition study. PwHD across the disease-span presented with social cognitive, executive and speed of processing deficits.

11.2.7.1 *Social cognition findings in context*

The new social cognitive tool, the ACS-SP, demonstrated similar discriminatory ability in PwHD to the widely used RMET, evidencing its utility in this population. Although the RMET and affect naming subtests did not show group level differences in preHD at baseline, contrasting to the literature^{354,356}, they were robust in manHD. Longitudinal patterns of decline were evident across timepoints, and performance was related to CAP100-disease progression estimates in preHD and across PwHD, supporting their use as longitudinal markers and, tentatively, **Hypotheses 5.2-5.3**. Absence of group differences at baseline do not offer evidence for **Hypothesis 5.1**, with only 7.9% of preHD scoring abnormally on the RMET, compared to 69.2% of manHD. Emerging signals over timepoints, not evident at baseline underscores the importance of longitudinal assessment in HD.

Associations here and in other studies also to functional scores (UHDRS) implying a linear deterioration of social cognitive function with disease progression^{228,336}, but the lack of harmonisation and over-reliance on the RMET in the absence of normative

correction for demographic variables complicates interpretation and cross-study comparison.^{358,364,366,692}

Although not compared directly to a ToM task, the RMET did not show a distinct factor from the ACS-SP affect recognition tasks in the factor analysis, an indication of insufficient construct and discriminant validity as a test of affective ToM and mirroring findings of similar analyses.⁶⁹² One study of patients with alexithymia and Autism Spectrum Disorder ASD⁸⁰⁰ found that although ToM tasks differentiated the groups, both groups were equivalently impaired on the RMET. Emotion recognition rather than ToM most likely underlies RMET performance, nonetheless, it remains sensitive for the assessment of social cognitive function in HD^{358,801} and ALSFTSD.^{548,685} Its interpretation as a test of ToM may need revision however. ROC analysis in this study found an optimal cut-off for impairment on the RMET to be $z = -0.768$, resulting in excellent sensitivity and specificity (both $\geq 84\%$). This is useful for characterising impairment in the absence of published normative cut-offs, though further validation in a larger sample is required. Optimal thresholds were also determined for the ACS-SP Affect Naming and Prosody-Face Matching.

The RMET and Affect Recognition subtests were strongly associated with verbal ability, but Prosody-Face matching was relatively independent. Contrary to **Hypothesis 6.1** this did not account differences above the group level, nor did a threshold emerge below which performance is adversely affected, when controlling for premorbid IQ. Nonetheless, to improve the sensitivity, especially clinically, the development of stratified normative data for the RMET, adjusted for education, age and sex is warranted.⁷²⁷

The strong relationship between the RMET and ACS-SP subtests indicate suitability for assessment of affective recognition in clinical and research contexts where the RMET is used. Demonstrating similar construct validity and sensitivity in HD, the ACS-SP Prosody-Face matching task showed the least involvement from verbal abilities, placing it as a valuable and quick task for screening affect recognition across populations of linguistic ability. The ACS-SP may have fewer limitations than the RMET, and thus be a more valid representation of an individual's social cognitive function across domains and levels of difficulty. An additional benefit to the ACS-SP battery is that it has published demographically-adjusted normative data.

Not analysed here, effect sizes across studies are highest for recognition of negative emotions (e.g.: anger, disgust, sadness) in PwHD.^{358,359,674} Isolating the negative emotions on the Affect-Naming and Prosody-Face Matching subtests of the ACS-SP might

improve sensitivity in the premanifest phase. As emotional prosody also showed the least association to verbal ability in hierarchical regression models, it is a superior candidate for use in preHD.

By linking ToM with motivational and behavioural phenotypes like apathy (**Chapter 9**), this thesis aimed to bridge social cognition and neuropsychiatry in HD and enhance the understanding of network-level dysfunction beyond the motor system in HD. Hendel et al.⁵⁰⁰ demonstrated this association with unidimensional apathy, but the absence of evidence in the current studies may be caused by a floor-effect, with low levels of emotional apathy in this relatively well manHD sample.

There are neuroanatomical distinctions in affect recognition and ToM domains whereby affective ToM is linked to limbic and right frontal structures; cognitive ToM relies on cortical midline and temporoparietal regions above that of orbitofrontal and fusiform.^{133,364,365,388} Both of these systems deteriorate progressively in HD^{503,802,803}, but neuropsychological studies have not differentiated these constructs sufficiently in HD. Nor was this possible to do in the current studies, and may require application of more valid tests of ToM. These results join a growing but inconsistent body of literature in HD social cognition.

11.2.7.2 Executive function in HD-Cognition

The results of executive function analyses showed reduced performance on all tasks for manHD, and on Digit span Backwards, processing speed (SDMT), and executive Inhibition (CWIT) in preHD. Forecast by **Hypothesis 5.1**, preHD scored intermediate to controls and manHD on these speeded tasks, indicating their sensitivity as cognitive biomarkers in premanifest/prodromal HD. Contrast scores isolating latent inhibition and flexibility constructs, extinguish all group-level effects, indicating impairments are largely attributable to slowed processing speed on these tasks, even in manHD, discussed above (**11.2.3.2.1**). Processing speed may not explain working memory performance however, which approached significance with a large effect size in preHD ($d=0.62$), almost perfectly intermediate to manHD. Nor does it explain increased error rates on executive tasks for manHD, which commonly present in later-stage HD at higher rates due to errors of executive monitoring.⁷²⁶

Error patterns revealed that individuals with manHD made significantly more errors than controls on the CWIT Inhibition task, but not on the SDMT. These findings suggest that although speeded executive performance is impaired, error monitoring

remains relatively preserved, particularly in preHD. This indicates a dissociation between neural circuits supporting processing speed and those supporting executive control in PwHD.⁸⁰⁴ Thus, while traditional timed measures highlight early dysfunction, task-monitoring and error metrics may provide complementary insights into specific executive subdomains affected in HD. This has been demonstrated in the HD-YAS study, in premanifest participants far from estimated diagnosis, which correlated with functional connectivity between the caudate and DLPFC and CSF NfL.⁴⁹¹

Demonstrated by this study is the magnitude of the effect speed can have on timed tasks of executive function in HD. Such tasks are good cognitive biomarkers^{228,345,392,494}, useful to differentiate PwHD from controls or track over time in clinical trials, for example.^{230,255,805} But from a cognitive-neuropsychological perspective, failing to acknowledge the speeded components, confounds construct validity and overestimates the magnitude of executive dysfunction in HD. Timed executive function tasks in addition to tasks of pure psychomotor processing speed (SDMT) may be redundant, as outcomes of the former are confounded by the effects of reduced processing speed. Appropriate control for the effects of speed by using error scores and contrast scores may provide better representation of the fundamental components affected.

11.2.8 *Aim 7: A new differential index score for ALSci: the RFI*

While it has been mentioned throughout this discussion, the particulars of the RFI should be addressed. Created on the principle of differential deficit and the discrepancy between phonemic and semantic fluency performance in ALS, this index theoretically isolates the selective impairment of this higher-order executive dysfunction in ALS, affording improved sensitivity and characterisation.^{16,118,624} There is evidence here for its discriminant validity across the ALS-relative and HD samples (**Hypothesis 7.2**). It successfully distinguished fALS relatives at baseline, both in Phase 1 and separately in Phase 2, with equivalent effect sizes. Divergent validity was provided by null findings in the HD-Cognition study (**Chapters 7 and 8**). It did not perform as expected longitudinally however. Practice effects differentially impacted fluency scores (semantic>phonemic), thus increasing the relative score, producing a less reliable result. As MND Family Study controls demonstrated in the endophenotypes chapter, control participants in HD-

Cognition improved significantly from T1 to T2 on semantic fluency, but not by the same magnitude on phonemic fluency, thus increasing the relative difference on the RFI.

According to the differential deficits theory, standardised RFI scores are less influenced by individual differences and could allow valid classification of ALSci even when raw phonemic fluency scores appear preserved, such as in individuals with high premorbid IQ.^{151,806} While full neuropsychological assessment is ideal, the RFI may serve as a brief marker of differential impairment in pwALS by the Strong criteria¹⁶, particularly when controlling for IQ, mood, or other confounds is not feasible.⁶²⁴

The Verbal Fluency Index (VFI) proposed by Abrahams is widely used to control for verbal motor impairments, but does not control for age, education, or premorbid ability. The RFI by comparison uses published normative data⁵⁶¹, stratified by age and education to isolate the specific impairment in ALSci, and as a result, also controls for verbal motor impairment, by factoring out semantic fluency performance. Further validation in larger samples is needed, as current findings suggest it may not yet meet reliability thresholds for use as an individual-level marker of network dysfunction, particularly over time. If successful, it may be of significance to research and clinics as an ALS-specific cognitive biomarker.

11.2.9 *Aim 8: ECAS-HD: cognitive screening in HD*

Chapter 10 presented data which showed the ECAS, widely used in ALS, has similar predictive value in HD. Both versions (A and B) showed excellent convergent validity with the gold-standard neuropsychological test battery. Verbal fluency emerged as the most robust and sensitive ECAS domain across ECAS A and B, with perfect sensitivity and NPV at both timepoints. Conversely, the visuospatial domain had poor sensitivity despite perfect specificity.

In the longitudinal analysis, LMMs controlling for premorbid IQ, found stable group differences over a 20 month median interval on all ECAS domains for manHD, and on Fluency, ALS-specific and ECAS Total domain scores for preHD, compared to controls. Scores did not deteriorate over time in the models, with the exception of Fluency in manHD, but meaningful change was identified on an individual level with RCIs. 66.7% of manHD and 20.7% of preHD fell in the abnormal range on ECAS B, T2, with 21.1% showing significant decline by RCI.¹⁶²

The memory domain showed the highest effect size for manHD in the LMMs, controlling for premorbid IQ, concordant with the other study of the ECAS in HD.⁷⁷⁶ However, Carelli et al.'s⁷⁷⁶ study reported memory and the non-specific domain as having the highest levels of impairment in HD, ALS-specific domains had the highest rates of impairment and indicators of reliable change in the current study. The non-specific domain demonstrated significant practice effects with 18.4% of PwHD improving from A to B.

The particularly low sensitivity of the Executive domain of ECAS A is discouraging. This was improved by Youden's J AUC generated cut-off (31.5), which may be more specific to HD. While the ECAS performs well in ruling out impairment, its use as a stand-alone tool to confirm domain-specific dysfunction may be limited due to reduced reliability for subtests and subdomains. However, this is also cautioned in the initial ECAS validation paper.¹⁶³ The balance of high NPV and acceptable-to-high sensitivity in key domains (ECAS Total, ALS-Specific, Fluency and Memory) are almost equivalent to the initial ECAS validation, and superior to those reported in the Italian study.⁷⁷⁶ Together, findings support the ECAS's use as a cognitive screening tool in HD clinics, and **Hypotheses 8.1-8.2.**

11.2.9.1 ECAS-HD considerations

The ECAS social cognition subtest (under the executive domain) has demonstrated poor performance in identifying faux pas recognition deficits, with sensitivity as low as 14% in prior work.⁷⁷⁸ Many recognise the value of social cognitive evaluation in HD, and recommend that it form a key part of the assessment of HD^{310,358,364}, and across neurodegenerative disease.³⁶⁶ Given the predominance of social cognitive impairment early in the HD disease course³⁵⁶, and indeed ALS¹²⁹, a reliable screening test should be used.

Reduced speed of processing on the SDMT is the most widely used and research-backed cognitive biomarker for HD^{255,392}, now also employed by research staging and prognostic models.^{256,313} The ECAS does not include tests of processing speed, or speeded tasks in general. Taken together with the findings from the HD-Cognition studies (**Chapter 7** and **8**) and HSG working group recommendations, tasks of cognitive processing speed should be assessed at screening.³¹⁰ This is a limitation of the ECAS.

The Fluency domain of the ECAS in **Chapter 10** showed perfect (1.0) sensitivity and NPV for ECAS A and B. It was the only ALS-specific task in the ECAS which premorbid

IQ estimates did to account for a significant proportion of the variance in scores. Although not declining over time in preHD, it demonstrated sensitivity and a significant interaction effect for manHD longitudinally, which may have been captured in ALS-specific RCI declines. Verbal fluency has been found to load more on processing speed than executive or linguistic ability in healthy and neurodegenerative disease populations.⁶⁹³ Although not directly psychomotor processing speed, the excellent classification accuracy and convergent validity of the Fluency domain on the ECAS may therefore capture the construct of this early cognitive biomarker in PwHD, and prove sufficient for screening purposes. Future iterations may benefit from restructured domain models and expanded content in underperforming subtests, particularly those assessing visuospatial function, inhibition and social cognition.⁷⁷⁸ The ECAS may be supplemented with a psychomotor processing speed task, potentially to create ECAS-HD. As a validated cognitive screening tool, ECAS-HD could assess baseline cognitive function and sensitively track meaningful longitudinal change, supporting early detection of and timely referral to appropriate clinical services. As in ALS, its widespread use could facilitate multi-centre collaborations and evaluation of cognitive function as secondary endpoints in clinical trials. In addition, although dysarthria is often a late and end-stage symptom of HD, having validated tools for assessment of cognition for this cohort is beneficial, given the paucity of similarly placed tools.

11.3 Discussion: Considering early cognitive & behavioural impairment in neurodegenerative diseases of the motor system

This thesis presents data that support two seemingly competing perspectives: it advocates for reducing reliance on eponymous disease classifications in clinical care by favouring a transdiagnostic approach to assessment and phenotyping; on the other hand, it emphasises the value of stringent phenotypic sub-classification and endophenotyping, to advance basic research, genetic and biomarker discovery, and clinical trial design. In the era of personalised and precision medicine, it is an attractive idea to be able to address mutations producing a specific endophenotype which forms part of a larger syndrome or disorder.

Eponymous classification has long been helpful in disorders to delineate pathology, symptomatology and prognosis. A balance of clinical utility with mechanistic understanding of conditions where macro-diagnostic categories are insufficient for prognostication and evaluation of new treatments in ALS and HD is required. Ensuring validity while characterising cognitive change over time through the prodromal phases in both ALS and HD will afford a better understanding of the heterogeneity and nature of developing cognitive impairment, and disease onset.

This approach also reflects a broader movement in neuropsychiatry towards dimensional frameworks, such as the Research Domain Criteria (RDoC)³⁹⁹, which prioritise mechanisms and domains over traditional diagnostic labels.⁴⁰⁰⁻⁴⁰⁴ The RDoC allows symptom mapping irrespective of nosological boundaries, particularly useful for diseases with overlapping phenotypes. This dimensional neuroscience approach shares promise for the characterisation and phenotyping of heterogeneous neurodegenerative disorders.⁴²⁵ Whilst an endophenotypic perspective will support genetic studies in ALS, similar methodologies are unlikely to apply to HD. Research domain criteria-type classification may support clinical care and possibly trial stratification in both disorders.

Timing of intervention is critical in neurodegenerative disease. Reliable biomarkers of surrogate endpoints in the premanifest phase of HD have long been sought.²⁷⁹ This is becoming more evident in ALS, with *The Lancet Neurology* series conveying a shift in the field toward preventive intervention, biomarker-led phenoconversion prediction, and stratified approaches to clinical trials.^{51,61-63} Appropriate use of cognitive screening measures, with tests sensitive to early stages of disease (phonemic verbal fluency in ALS and processing speed and affect recognition) are needed

for evaluation of extramotor symptom treatment responses. The ECAS may provide such repeatable markers in both disorders.

11.3.1 *Premanifest cognition & non-motor diagnostic criteria*

Inclusion of the SDMT in the HD-ISS²⁵⁶ and PIN_{HD}³¹³ are a sign of a growing recognition of the cognitive and behavioural phenotypes that can present in advance of HD-clinical diagnosis. With the shift toward biomarker-based diagnostics, neuropsychological tools have a role in early detection, differential diagnosis, and disease tracking. Despite evidence of social cognitive impairments in many neurodegenerative diseases, including early-stage HD and ALS/FTD^{115,129,133,364,365,375,807,808}, their translation into clinical neuropsychology has been limited. The *SIGNATURE* initiative³⁶⁶, across diagnostic categories for NCDs in general, and the HSG Neuropsychology Working Group Neuropsychology³¹⁰ for HD in particular, seek to harmonise standards in assessment of social cognitive function. Emphasising the importance of these initiatives, the HD-Cognition study in **Chapter 7** revealed functional impairment in clinically asymptomatic preHD participants. preHD endorsed significantly higher levels of fatigue and difficulty participating in society, with increases at the trend level in other areas of disability on the WHODAS. Companions also rated them higher than controls on subtle changes to behaviour, compared to 10 years ago. Research presented in this thesis supports the refinement of assessment methods and diagnostic criteria in HD^{307,308,310}, which may extend to NCDs more generally³⁶⁶, to define clinical recommendations and produce validated assessments of social cognitive function.

11.3.1.1 *Increasing power in “presymptomatic” studies*

Methodological and conceptual issues hinder “presymptomatic” research in ALS in general. Inter-individual variation in cognitive performance is high in the general population.^{296,297} It is higher still in heterogeneous disease groups spanning premanifest, prodromal and manifest disease. Models across the studies in this thesis show large subject-specific random effects, even after controlling for covariates.⁶²¹ This emphasises the importance of LMMs in research and RCIs in clinical practice, which control for this variability, in evaluating longitudinal cognitive performance.^{606,809}

There are limits to test sensitivity and statistical power without fully penetrant/diagnostic genetic markers which can also facilitate stratification due to associations with disease progression, as in HD.^{100,215,465,810,811} Even with such markers in

HD, the premanifest phase poses challenges to researchers, clinicians and trial runners. Nonetheless, improving our understanding of the presymptomatic and prodromal phases of *C9orf72*-ALSFTSD using cognitive biomarkers can help develop more accurate models of prognosis and support the evaluation of novel therapeutics during this critical window for intervention.⁴⁵⁹ Multimodal approaches integrating cognitive assessment with neuroimaging, electrophysiological and fluid biomarkers (NfL) will offer the most promise for insight into the pathogenesis and evolution of cognitive change in ALS and *C9orf72*-associated disorders.^{464,468,485} This integrative strategy may enable the development of reliable biomarkers for presymptomatic detection and disease monitoring.

Migliore et al.³⁹⁰ recently used the Enroll-HD dataset to compute Composite Cognition Scores (CCS). CCS demonstrated superior predictive ability, whereby lower scores prior to motor symptom onset were predictive of more rapid motor symptom progression, independent of CAG-repeat length. In a related study, Martinez-Horta et al.³⁸⁹ categorised participants in Enroll-HD as slow and fast progressors, based on cognitive change scores. 1-year decline on Enroll-HD cognitive scores predicted faster deterioration on cognitive, functional, and motor measures, improving predictions than baseline scores alone. As with apathy³⁸⁷ and social cognitive tasks⁴⁷⁰, CCS and slopes of change could aid early prognostication, guide pre-symptomatic intervention, and enrich clinical trial samples in concert with CAG-associated predictors in preHD.^{389,390}

Whilst cognitive composites might provide predictive power in HD, they are unlikely to do so in ALS, where more specific impairment is evident. Informed by theory, the RFI, discussed above, may however offer a sensitive and specific marker of ALSci, which has shown utility here in the fALS cognitive endophenotype and discriminant validity in the HD-Cognition cross-sectional and longitudinal analyses.

11.4 Limitations

11.4.1 *Impact of Covid-19*

The Covid-19 pandemic impeded research undertaken for this thesis. In particular recruitment for the HD-Cognition study, as well as the general impact to processes and procedures necessitated by restrictions.

11.4.1.1 Remote testing

The general limitations of tele-neuropsychology, including unstable internet connection and environmental distraction apply to the majority of the neuropsychological assessments performed. Research protocols aimed to minimise these effects. Conversely, the online medium may have had a slight positive effect on test results as participants may have experienced reduced interpersonal and performance anxiety than when face-to-face with examiners.⁶³³ In any case, the online validation study (**Chapter 5.1**) indicated sufficient reliability of online administration.

11.4.2 Genotype data

This cohort of asymptomatic C9-carriers is small. Missing genotype data for Phase 2 prospective relatives in the MND Family study as a result of Covid-19, limits the strength of the cross-sectional findings on *C9orf72*-associated factors. Inclusion of greater numbers of older asymptomatic *C9orf72*-positive relatives, may capture incipient cognitive change relating to disease manifestation, should it occur, highlighting the most sensitive cognitive biomarkers in early disease. Results from samples taken over the study period will supplement Phase 1 findings when available later this year.

Additionally, in the HD-Cognition study and the pwALS clinic based-study, participants were not included in subgroup analyses because clinical genetics data were not available, or in the case of HD, did not disclose the number of CAG-repeats. This limited power for these analyses.

11.4.2.1 Blinding to genetic status

Another limitation of both the HD-Cognition study and the MND Family Study, is the unblinded nature of the assessments. In HD, the gene status of each participant was known for clinically unaffected individuals. For relatives pwALS, although the individual participant gene status was not known to the researchers, family history (how many members of their family were pwALS), and in some cases, the genetic status of the proband was known. In both cases, although researchers took an unbiased approach to the administration and scoring of the neuropsychological assessment adhering to strict protocols and manual guidelines, this knowledge may have introduced unconscious bias.

11.4.2.2 Genetic mosaicism & somatic expansion

Genetic mosaicism and somatic expansion of the pathologically-expanded genes have been observed in both HD and ALS⁸¹², and may be the primary driver of pathogenesis.²⁷¹ Limited to peripheral blood samples, expansion size might vary in other somatic tissues, most operatively neural tissues. Clinical markers such as age-of-onset, phenotype and prognosis are therefore not related to the expansion that is most relevant and confounds research aiming to elucidate pathological mechanisms and evaluate treatments. The limitation of predictive CAG-related models should be acknowledged. This is less relevant in *C9orf72*-carriers where repeat expansion does not appear to relate to clinical characteristics.^{78,813} Pathological study of discordant C9-negative pwALS from C9-positive kindreds will help determine if discordance in genetic status is the result of alternative genetic causes, or somatic expansion to pathological levels in neuronal tissue, which is not present in blood.⁸⁰

11.4.2.3 Other data availability limitations

The absence of objective motor assessment in HD-Cognition limits somewhat conclusions about disease progression. Models, such as the PIN_{HD} integrate motor, cognitive, and functional markers to model disease trajectories, providing increased accuracy.^{313,319} The absence of motor and imaging data limits alignment with these international datasets, research staging criteria and constrains the generalisability of findings.²⁵⁶ HD-Cognition provides a rich repository of neuropsychological data on Irish PwHD however, which has much potential for future study.

11.4.3 Recruitment, attrition & engagement

Appointment attendance can be impacted by apathetic symptoms, and issues with planning and organising in real-world environments, often present early in HD.^{814,815} This resulted in many missed appointments, and some difficulty with contact and recruitment for all timepoints in the HD-cognition study in particular.

Missing data due to attrition, a common issue in longitudinal research, had been considered in a priori power estimations. These indicated a need for a larger sample than was ultimately achieved in the HD-Cognition study, particularly for the premanifest group due to smaller effect-sizes. Statistical power was therefore lower than intended. To mitigate this, linear mixed-effects modelling was employed to maximise the use of available data in the longitudinal design.

11.4.3.1 Recruitment bias & attrition

There were several levels of sampling bias in these studies. A number of eligible participants chose not to take part, resulting in the absence of complete families in the MND Family study. Relatives and patients declined participation due to an aversion to the testing procedures (e.g.: genetics, cognitive assessment), or other factors reducing their willingness to engage. Many pwALS did not consent for their relatives to be approached.

Estrangement is not uncommon in large families, particularly with neuropsychiatric family history. Many members of families were not contactable, and others, not willing to participate for various reasons. Some had withdrawn from social contact, a core feature of many neuropsychiatric illnesses. Such factors may themselves be linked to characteristics of interest, including personality traits associated with neuropsychiatric endophenotypes or neurodevelopmental effects related to C9+ genetic status, for example.⁹⁸

Recruitment bias and self-selection likely skewed the sample who completed the full battery compared to the ECAS only paradigm in the Pleiotropy study, and longitudinal timepoints in the HD-Cognition study. Sensitivity analyses were performed and reported in the relevant studies. Analysis of the participants who declined follow-up in both studies showed reduced T1 performance, older age and higher apathy scores. In clinical research attrition is often non-random, skewing results as well as affecting statistical power. Attrition of the most cognitively/behaviourally impaired compounds practice effects in longitudinal designs, with successive timepoints seeing scores increase. Balancing competing timelines to minimise practice effects and attrition and maximise effects aimed to address this as best as possible, but were limited by practical constraints. Even still, practice effects are evident over long periods in neuropsychological testing, and so controls are needed in longitudinal designs.^{536,537}

Those who declined participation entirely may have had more interesting cognitive profiles still, thus biasing the study samples towards lower severity of cognitive and behavioural symptoms. Attempting to minimise these effects in Phase 2 of the Pleiotropy study, the ECAS-only cohort were contacted alongside the longitudinal follow-up study which improved completeness and representation of the population in the extended neuropsychological assessment.

On the other hand, neuropsychological assessment is comprehensive, and therefore takes up to three hours. preHD and unaffected fALS relatives tended to be younger than retirement age, most of whom had full-time jobs and young families.

Scheduling and workload may have biased the sample away from those with regular work, for example. This is pertinent in family-based designs, neuropsychiatric assessment and for representativeness of longitudinal data. This self-selection bias was likely compounded by the longitudinal design, skewing further the follow-up samples. The online assessment medium mitigated this, and the examiners were flexible with scheduling to maximise recruitment potential.

11.4.3.2 Control sample bias

Control participants across all studies had relatively high levels of education and higher than expected premorbid and current IQ estimates indicating another level of self-selection sampling bias. Controls were mostly volunteers and the spouses of disease/disease-relative participants. Volunteer samples, as compared to random selection of control participants, tend to have higher cognitive performance, have higher education and are healthier overall.^{816,817} Matching and statistical control for potential confounders was practiced throughout (e.g., for premorbid IQ estimates).

11.4.4 Psychological effects of ALS/HD-family membership

The psychological effects which can potentially influence cognitive test scores in kindreds of neurodegenerative disease cannot be discounted. Anticipatory health anxiety, grief and engaging with research related to the hereditary disease may have impacted reported findings. Efforts were made to control statistically for psychological stress (anxiety/depression) where appropriate, and fALS relatives did not differ significantly from controls. Gene-negative and non-biological family members are included in the HD-Cognition study control sample to further minimise environmental effects.

In the MND Family Study, unaffected relatives were not in the acute phases of caregiving, often did not share a household with the proband, and in the Phase 2 longitudinal follow-up, had some distance from relatives with symptomatic disease who had passed away years before. Family aggregation studies find increases in psychiatric and other conditions in the distal family history also support the validity the ALS endophenotype findings.¹⁸⁹⁻¹⁹¹ Demonstrated directly here, **Chapters 5** and **9** show that although psychological factors may be related to cognitive function, they do not account for outcomes.^{646,649,818}

11.4.5 *Limitations of neuropsychological assessment*

Many of the variables presented in this these are influenced by biological, psychological and social factors, and do not occur in isolation. Complex conditions require careful and conscientious assessment. Significant effort was made to control for extraneous factors which may affect cognitive outcomes (e.g.: apathy, fatigue, mood, motor function), and multiple levels of investigation and complexity were employed to strengthen study validity. The benefit of screening tools, such as the ECAS and Distress Thermometer, offer predictive ability that streamline clinical services, and convenient benchmarks for research and trials.

11.4.5.1 Self- or proxy-reported behavioural measures

A reliance of self-report measures, even in the case of a healthy proxy/carer-report, introduces variability to measurement unrelated to disease. Control group comparisons facilitate interpretation for proxy-measures.

11.4.5.2 Unmatched group demographics

Age, education and premorbid ability are highly related to cognitive test outcomes.⁶⁵¹ Neuropsychological test scores were standardised to z-scores based on education- and/or age-matched normative data from published sources. This therefore represented participant's cognitive performance relative to their age/education-group. For tests where normative data did not stratify scores by age/education, missing factors were included as predictors in regression models, enabling assessment whilst controlling for differences in demographics, such as age.

Although TOPF-FSIQ is theoretically stable over time as a measure of premorbid IQ, it is an estimate based on reading/pronunciation of words and as a result, can be associated with neurodegenerative disease progression, demonstrated here in the manifest group in HD-Cognition. Controlling for premorbid IQ estimates in such cases dampens the effects of absolute group differences on cognitive tasks. This was somewhat addressed by using baseline TOPF-FSIQ scores across testing intervals in longitudinal designs, and investigating the independent effects of education, a commonly used demographic control for ability.

In **Chapter 6**, fALS relatives have lower premorbid IQ estimates, but are matched to controls for years of education. Reduced TOPF-FSIQ in this case may be the product of underlying network abnormalities, rather than a demographic confound to control for.

Applying Crawford's method^{526,624}, typically used for elucidating differential deficits in cognition in neurodegenerative disease, intra-individual variability between specific tasks with shared cognitive requirements can accentuate construct-/task-specific impairments relative to general performance, as is shown with the RFI.

11.5 Future directions

This collection of studies has highlighted issues and made recommendations for the assessment of neuropsychological symptoms in early neurological disease. In response to the RMET sharing factors of affect recognition with the ACS-SP subtest, alternative tests are warranted to probe valid measures of ToM in HD. Other social cognitive assessments such as the Mini-SEA and TASIT may offer more insight into the particular ToM difficulties emerging in HD.^{353,362,819}

Performance on the SDMT is the most widely used non-specific cognitive biomarker of decline in preHD. Taking insights from Multiple Sclerosis research, where the SDMT is also a sensitive marker of cerebral dysfunction³⁴⁷, combined with the current findings from the longitudinal HD-Cognition study, alternative forms of the SDMT are required to minimise practice effects and maximise the validity of repeat scores.⁸²⁰ Large studies such as TRACK-HD and Enroll-HD which use the SDMT as a landmark clinical biomarker would benefit from the introduction of alternating versions, which show excellent reliability and equivalency.⁸²⁰

Addressing modifiable risk factors throughout life, such as continued education, abstinence from smoking, low-to-moderate alcohol consumption, and weight management have been shown to be associated with delayed time to disease onset in the preHD population.⁸²¹ This not only has a benefit to cognitive symptoms, by increasing cognitive reserve, but also in delaying pathology, and in turn motor and psychiatric symptom onset in HD. Ongoing work in our group assessing frailty as a moderator of disease onset could highlight targetable modifiable risk factors in the premanifest stage.

11.5.1 *Methodological contributions: test validation, standardisation & control for additional factors*

Introduced to quantify subjective distress and evaluate procedures for ECAS administration in the MND Clinic, the DT identified high levels of distress in pwALS. Further validation is required. Updated thresholds and an ALS-specific DT 'problems list', created

to suit common challenges and sources of distress in pwALS, would facilitate signposting through clinical services, e.g., social work and psychology.⁶³⁹

The online neuropsychological assessment has shown reliability in **Chapter 5**. This online assessment battery could lower the barrier to patients in need of clinical neuropsychological input but who cannot travel, for example. Adhering to developed protocols, it is a valid alternative. Remote versions of the ECAS were also used throughout these studies, and have shown reliability and feasibility in a study from the authors⁵⁹⁵, also providing remote monitoring opportunities to clinicians in ALS, and HD.

A key next step for the RFI will be to establish reliable clinical classification thresholds using large, demographically stratified normative data. This could quickly and easily be applied to retrospective ALS databases, with evaluation of performance over time, to investigate the limitation of practice effect confounders, discussed above. ROC analyses could determine optimal cut-offs for ALS-specific impairment, comparable to methods used for ECAS validation.^{16,163} Standardising RFI scores in this way would enhance its clinical utility as a brief and sensitive marker of selective fluency deficits in ALS.

11.5.1.1 Harmonising cut-offs

The use of age- and education-stratified normative data for valid interpretation of the ECAS is emphasised by the strong associations shown in the current studies.^{163,770} Years of education has been increasing in the general population.^{822,823} In Ireland, the majority of people complete secondary level education and third level education, with only 3% of 25-64 year-olds finishing at primary level or below (≤ 12 years).⁸²⁴ The cut-off for determining 'low' education may be underutilised, reducing overall sensitivity.^{162,163}

Many centres using the ECAS do not use age- and education-stratified cut-offs, and there is variation in how impairment is defined.^{159,616,767,770,825} A re-validation of the clinical cut-offs for the ECAS is timely, as work is beginning to harmonise procedures and scoring of the ECAS under Precision-ALS{Costello, 2025 #30604}, to improve data comparability across international consortia and clinical trials. Such endeavours would also benefit further development of the ECAS-HD, which was shown to have high sensitivity and NPV using current cut-offs here in sample of PwHD (**Chapter 10**). Harmonisation on the definitions and categorisation of reliable change to scores over time also addresses issues highlighted in part two of **Chapter 5**, where interpretation of normality is not reliable without the use of Reliable Change Indices.¹⁶²

Further to this, a minimum viable and validated battery which adheres to the Revised Strong Criteria¹⁶ would support the rapid, but comprehensive categorisation of patients for clinical diagnostic and prognostic use, providing more reliable measures than the ECAS, as well as improving dataset compatibility across sites. It would also allow rapid adoption and validation of the RFI.

The BBI has shown excellent psychometrics in ALS-FTD spectrum disorder^{153,578}, and has recently been translated into Italian and Chinese.^{826,827} However, it was created and validated prior to Strong's 2017 criteria for ALS-FTSD and does not offer domain scores aligning it with these criteria. Development of criteria-based scales would renew the utility of the BBI as a gold-standard tool for diagnosing ALSbi, facilitating its use in clinical trials and international consortia.

11.5.1.2 Practice effects

Practice effects were common in the HD-Cognition study (**Chapter 7** and **8**). Whilst their effects are long recognised in neuropsychology^{537,828}, many large cohort studies do not have procedures in place which systematically control for them, despite repeated visits (e.g.: Enroll-HD, TRACK-HD, PREDICT-HD, GENFI). These large platforms provide excellent opportunities for standardisation of methods which maximise validity of repeated measures, such as alternate forms, RCI, or regression based methods. Creation of demographically-matched normative data is important, as recently published in the Enroll-HD study⁸²⁹, but as highlighted in **Chapter 5** the application of normative cut-offs without recognition of practice effects and non-disease-related variation in test performance obscures meaningful change and categorisation in longitudinal assessment.

Work here highlights the significance of social cognitive function, particularly affect recognition, as a marker of cognitive impairment. Social cognitive tasks are not included as part of the cognitive battery within Enroll-HD. With Enroll-2.0 on the horizon⁸³⁰, findings here add to the neuropsychological research advocating for improved measures of the early-HD extramotor phenotype. Standardisation of measures and procedures for interpretation will allow collaboration in international consortia and larger multi-site cohort studies such as GENFI⁶⁵ and Enroll-HD^{538,539}, aiming to examine the trajectory and presymptomatic profile of at-risk individuals.

11.5.1.3 Additional avenues to pursue in HD-Cognition

There is a wealth of data collected within the HD-Cognition study which was not analysed as part of this thesis. These include exploration of the constructs underlying verbal memory recall in preHD using improved error and contrast analysis on the CVLT-III⁴⁹⁵, caregiver data, and continued collection and analysis of longitudinal cognitive and behavioural data presented here. Increased sample size would permit a more generalisable interpretation of the ACS-SP battery, which was limited here.

While the focus of the research on executive function has highlighted the confounding effects of speed of processing in HD, there is still much scope to determine specific latent constructs that reflect discrete fronto-striatal network impairments. Identifying and validating such constructs may improve the sensitivity of cognitive assessment tools and offer clinically meaningful markers of pathological spread, analogous to Braak staging in Alzheimer's disease. This approach mirrors recent findings in ALS, where regional TDP-43 pathology correlated with domain-specific cognitive deficits during life.⁵² In HD, this could support the development of cognitive staging models and more precise phenotyping of executive dysfunction across the disease continuum.

Environmental and psychological factors such as frailty, cognitive reserve (CR) and apathy may also moderate the manifestation of clinical heterogeneity in terms of onset and progression of cognitive impairment in ALS and HD.^{174,831} One study by Bonner-Jackson et al.⁸³¹ assessed the effects of CR in preHD, finding less striatal pathology and slower cognitive decline as they approached diagnosis. Further analyses of CR data within HD-Cognition can assess its impact on longitudinal cognitive profiles as another potentially modifiable risk factor for improved wellbeing.

Evidence for distinct extramotor phenotypes in HD is lacking. Multi-modal techniques are necessary to evaluate presymptomatic disease progression, which should include motor, cognitive and behavioural measures.²⁵⁵ Cognitive dysfunction has been associated with functional imaging markers in HD^{726,832}, including a small study which showed electrophysiological network dysfunction during memory performance.⁸³³ Many preliminary prognostic and candidate electrophysiological biomarkers have been proposed in pwALS.^{183,531,834-836} Application to C9orf72-positive carriers aiming to elucidate early markers of disease progression is ongoing, and providing promising results.¹⁸⁴ The same methods may prove useful in determining electrophysiological correlates of the cognitive endophenotypes in unaffected fALS relatives in general, complimenting and

extending the results presented here. So too would investigation of the electrophysiological correlates of cognitive performance in HD-Cognition benefit PwHD, through biomarker development and prognostication.

11.5.2 “Predictive” genetic testing & clinical genetics

With increased understanding of genetic mechanisms in ALS and the possibility of disease-modifying and potentially preventative therapies for ALS, genetic counselling in routine clinical care is increasingly relevant. In Ireland, this is most relevant to *C9orf72*. Gene pleiotropy and reduced penetrance of *C9orf72* have hindered implementation of presymptomatic testing services in Ireland. Despite this added uncertainty, the demand for presymptomatic testing remains, with some studies showing higher demand and follow-through for persons at-risk of C9-ALS/FTD, compared to HD population, despite full penetrance.¹¹⁰ Whilst it was not possible to perform the study, preliminary groundwork by CP indicated a clear interest and unmet need for presymptomatic genetic testing among at-risk individuals in Ireland. Established research from other centres performing predictive testing for *C9orf72* will support creation of these services in Ireland along best-practice guidelines.^{112,458,837,838} Grassano⁴¹⁵ and Benatar et al.^{468,839} provide commentary and a proposed framework for integrating genetic risk into ALS/FTD care. There are many limitations and restrictions to this approach, but as research progresses, a patient-centred, ethically robust, and scientifically grounded approach is essential, much of which can be gleaned from the experience of clinicians and researchers in HD.²³⁷

In addition, personalised penetrance estimation of *C9orf72* have high potential for facilitating risk-profiling and complex decision-making in clinical genetics services.⁸⁴⁰ Predictive clinical genetic testing in ALS would also accelerate research and improve clinical trial participation, as it has done in Huntington’s disease, allowing evaluation of new therapies from early in the disease course, critical to preventative intervention.

11.5.3 Endophenotypic heritability

Presence of cognitive endophenotypes might suggest that the endophenotype and possibly disease phenotype could be heritable in ALS patients. The heritability of clinical phenotype has not been studied in fALS, largely due to the small number of cases with phenotypic data available in parent-child dyads. Ryan et al.⁴⁷ performed a study using data from the Irish MND Register examining heritability in such a population. They

report that children of pwALS who themselves develop the condition, manifest 17.2 years earlier than their parent. On average, pwALS with a parental history had onset 7.2 years before those without.⁴⁷ Additionally, 21.9% of the parent-child dyads in this study had cognitive-onset ALS. This proportion is larger than the usual incidence of cognitive-onset ALS. However, 60% of this sample were *C9orf72* positive, which perhaps explains this finding.⁴⁷ Earlier onset and prominent cognitive features at onset may evidence a higher genetic burden in parent-child dyads and in ALS kindreds.

Should the endophenotypes uncovered by Costello²¹⁰ and Ryan²¹¹ in at-risk ALS kindreds continue to be replicated and validated, as they are in this thesis, sub-phenotypic presentations may be commoner within families (e.g.: ALSn vs ALSc/bi/FTD, limb- vs bulbar-onset). While AoO has shown increased heritability between parent-child dyads⁴⁷, broader clinical phenotype has not demonstrated heritability. Examining patient-relative linkages on these endophenotypes in fALS cases would further shed light on the heritability of extramotor symptomatology, in addition to extending the evidence for segregation of the endophenotype within families. Direct assessment of the heritability of cognitive endophenotypes is a necessary step towards ratifying putative cognitive endophenotypes of ALS. Additional research is needed to examine the extent to which candidate cognitive endophenotypes in pwALS-probands are associated with performance in their kindreds. Models in **Chapter 6** indicated that portions of variance were explained by family membership across many variables, however this may also be true in control families where genetic and environmental factors contribute. Evidence of a more systematic relationship in kindreds, above that of controls, would further support candidacy and genetic aetiology of the putative cognitive endophenotypes for ALS.

11.5.4 *Markers of endophenotypic strength & risk within ALS kindreds*

Current uncertainty around risk and prognosis due to reduced penetrance and pleiotropic effects make genetic counselling and clinical decision making difficult for professionals and carriers. Penetrance is highly variable between families harbouring the *C9orf72* repeat expansion, ranging from 20 to 80%.^{72,75,77} Personalised penetrance estimators could also prove useful scientifically. Within-family penetrance estimates could be leveraged as a biomarker in support of exploration of modifier genes and endophenotypic strength.⁸⁴⁰ It is reasonable to expect that families with higher

penetrance estimates and a higher disease liability would display a higher burden of endophenotypic traits associated with the ALS phenotype. Integration of measures for a multimodal approach may uncover modifier genes of *C9orf72* pathogenesis and phenotypic expression, consistent with the oligogenic and multi-step hypotheses of ALS.

11.5.5 *Recommendations for research in ALS kindreds: endophenotypes & presymptomatic C9orf72*

We should continue to evaluate the isolated effects of *C9orf72* on phenotypic and sub-phenotypic traits in ALS and ALS kindreds. However, in the context of its low and variable penetrance^{75,77}, gene pleiotropy^{191,192}, and the substantial unexplained heritability of ALS⁴⁷, as well as the presence of discordant *C9orf72* families⁸⁰, endophenotypic traits should be assessed alongside presymptomatic and phenotypic studies of *C9orf72*. ALS is an oligogenic disorder and other unidentified genetic variants may influence phenotype/endophenotype expression within these families and thus highlight the need to reconsider study and trial design. To better delineate the unique and shared characteristics of gene carriers and non-carriers, *C9*-negative relatives may not be suitable as the sole control group. Future studies should include both *C9*+ and *C9*- family members alongside an independent, population-based healthy control sample in studies and trials. With the development of new presymptomatic frameworks, growing demand for presymptomatic genetic testing, and the emergence of gene-targeted therapies, it is imperative that we deepen our understanding of genetic risk within familial ALS kindreds, beyond just *C9orf72*.

11.5.6 *Other potential endophenotypes: Neurological soft signs*

Another relevant area of investigation from the endophenotype literature introduced in the first chapter are neurological soft signs (NSS).²¹³ NSS are particularly promising as endophenotypes in motor-system-disorder neurology as they may evidence general neurological dysfunction, or traits manifesting as subtle motor or sensory abnormalities. Assessment of NSS in fALS relatives presented in the current MND Family study is underway. Whilst the inclusion of NSS data is beyond the scope of this thesis, it is a promising avenue that will undoubtedly supplement the existing research on cognitive endophenotypes in ALS, as it has done in Schizophrenia research. A multimodal approach to endophenotyping, integrating NSS and neuropsychological traits, has been proposed

and may well increase power for identification of at-risk relatives²¹⁴, further supporting genetic studies in ALS.

11.5.7 *Combination of cognitive & other measures*

The studies presented in this thesis had a strictly clinical neuropsychological focus. Mentioned in the introduction, findings support the value of combining fluid biomarkers with sensitive cognitive and neuroimaging markers to define and track the preclinical stages of HD and C9-ALS. Large scale multimodal studies such as the premodiALS⁸⁴¹, Enroll-HD⁸⁴² and HD-YAS within TRACK-HD^{316,492}, are required to uncover early clinical and molecular markers by combining clinical assessments with multi-omics profiling in individuals at pre-symptomatic and early symptomatic stages. Reliable biomarkers for the premanifest stages of HD and ALS will enhance early diagnosis, facilitate clinical trial readiness, and inform targeted therapeutic strategies.

Multimodal approaches to ALS endophenotyping, integrating the cognitive traits identified in this thesis with behavioural measures, neurological soft signs, and structural or functional imaging offers a promising path toward capturing the complex genetic architecture of the disease. Composite-endophenotype frameworks offer the most promise to enhance the power of future genetic studies aimed at uncovering novel ALS risk genes. Similarly, future research combining cognitive biomarkers, neuroimaging, fluid biomarkers, and computational modelling may help clarify the mechanisms driving divergent disease trajectories in HD. A recent publication using Dynamic Time Warping based unsupervised clustering across multi-modal data has demonstrated the possibility of such multimodal computational methods to accurately predict disease trajectories by their time-patterns.⁸⁴³ Such models need large and consistently standardised datasets, which the HD community are well placed to benefit from with studies such as ENROLL-HD, PREDICT-HD and TRACK-HD. Large initiatives in Precision-ALS is a step in the right direction towards this possibility in ALS, which would benefit from the lessons learned in HD.

11.6 Conclusions

This thesis has presented findings from transdiagnostic neuropsychological assessments across time and various stages of risk and disease with a view to highlight commonalities and distinctions with implications for clinical practice and neuropsychological theory. Disambiguating diagnostic categories is necessary in the era of precision medicine, where sub-classification must occur to properly evaluate the efficacy of emerging targeted therapies. Deep phenotyping and clustering of cognition in preHD and unaffected *C9orf72*-carriers can support this work and provide insights into pathogenesis and evolution of symptoms from asymptomatic gene-carrier through manifest disease. The studies presented here have provided an in-depth examination of cognitive function across profiles of risk and stages of disease in ALS and HD.

An independent sample unaffected fALS relatives demonstrated cognitive trait differences, along with evidence for stability of these characteristics over time by longitudinal follow up over 4-6 years. Together with the initial findings, there is strong support for continued pursuit of these traits as cognitive endophenotypes of ALS, with the ultimate goal to further unravel the pathological mechanisms of ALS beyond the known gene-variants.

Findings from the HD-Cognition study underscore that cognitive decline, apathy, and social cognitive dysfunction are not late byproducts of HD, but rather core features that emerge early from the same network pathology driving the disease. This has significant implications for how we detect, monitor, and ultimately treat HD. These results strengthen the case for expanding HD diagnostic criteria beyond motor signs to include cognitive decline. In practice, routine neuropsychological screening could enable earlier identification of HD-related cognitive changes and prompt interventions well before significant functional decline. The validation of tools like the ECAS in HD is particularly valuable. It demonstrated high convergent validity and proved sensitive to the HD cognitive profile. It's widespread use in ALS, with serial forms, different language versions and availability of robust normative data and clinical cut-offs mean can be readily implemented in HD clinics. Likewise, incorporating social cognition measures in assessments is recommended, since this work confirms that impairments track disease progression and impacts daily living.

Longitudinal studies with refined neuropsychological assessments promise to further elucidate the prodromal cognitive and behavioural changes in ALS and HD. More

broadly, this thesis advocates for a dimensional, transdiagnostic approach to neurodegenerative diseases. By comparing HD with ALS, it reveals overlapping patterns of network dysfunction, evidenced by shared apathy subdomains pointing to common underlying fronto-striatal circuit pathology in motivation and executive function. Such insights encourage moving beyond siloed disease labels toward mechanism-based classifications, such as the Research Domain Criteria.

In conclusion, this body of work affirms that comprehensive neuropsychological evaluation is indispensable for early detection and nuanced understanding of HD and related disorders. Characterising cognitive and behavioural changes in the preclinical stage enriches our models of disease progression, opening new avenues for intervention. By integrating these neuropsychological markers into clinical practice and research, we can improve patient care – tailoring support in the early stages – and enhance clinical trial design by identifying at-risk individuals and relevant outcomes.

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Appendices

Appendix 1 Non-motor diagnostic criteria for HD (Chapter 1: HD)

Proposed non-motor diagnostic criteria from Considine et al. (2025)

1. Asymptomatic HD:

- a. CAG expansion confirmed.
- b. Absence of neurologic symptoms/signs and/or neurobehavioral syndromes.

2. Possible symptomatic HD:

- a. CAG expansion confirmed.
- b. Absence of unequivocal motor signs of HD on neurologic examination.
- c. Presence of nonmotor neurologic symptoms/signs and/or neuropsychiatric syndrome(s) that are:
 - i. Evident on initial clinical assessment.*
 - ii. Change from baseline and persistent/ recurrent per history (patient/informant).
 - iii. Not better accounted for by alternative neurodegenerative diseases, nondegenerative medical disorders, or primary psychiatric diagnoses.#

3. Probable symptomatic HD:

- a. CAG expansion confirmed.
- b. Absence of neurologic symptoms/signs and/or neurobehavioral syndromes.
- c. Presence of nonmotor neurologic symptoms/signs

*Per objective assessment measures, e.g., cognitive screen or neuropsychological evaluation (especially if cognitive symptoms are sole clinical feature), psychometrically validated symptom reports from patient and/or informant.

Clinically indicated medical diagnostics should be used to exclude alternative diagnoses.

^Longitudinal clinical assessment recommended over 3–12 months, or, sufficient time for therapeutic response to the clinical management provided.

Considine, C. M., Eddy, C. M., Frank, S. A., Kostyk, S. K., Oosterloo, M., Killoran, A., . . . Claassen, D. O. (2025). Improving the Clinical Diagnostic Criteria for Genetically Confirmed Adult-Onset Huntington Disease. *Neurology Clinical Practice*, 15(2). doi:10.1212/cpj.0000000000200427

Appendix 2 Online Risk Protocol (Chapter 3)

Prior to assessment / after the participant has completed the online 'Participant' questionnaires [3 HD-Cog Questionnaires - ...]:

- Review questions for risk
 - o **NB:** PHQ-9 – Q9
- If risk identified in questionnaires, print off and highlight for discussion with the patient at the time of the remote assessment.
- If no risk identified, note same on research cover sheet that the questionnaires have been reviewed.

Online Assessment:

If a person discloses information about suicidal ideation or intent during an assessment and you feel they may be in immediate danger to themselves or others – **complete a risk assessment** to clarify their thoughts and feelings of what they have disclosed

- **Actively listen** and **remain calm** in the situation - this will help to provide assurance that is necessary to support the individual
- Remind participant of **limits of confidentiality** (i.e., if they disclose information that is a cause for concern, you must relay this information on to a Senior Psychologist)

RISK ASSESSMENT:

1. Ideation – Identify whether the individual has thoughts about harming themselves/ that they would rather not be here any-more.

IF YES – Identify how frequently do they have these thoughts?
Are they worried/distressed by these thoughts?

2. Intent – Identify whether the individual intends to act on these thoughts

IF NO - Emphasise that though these thoughts are frightening, they can be
normal
reactions to psychological distress

Encourage individual to go to **GP** if they become worried about these thoughts
or

Encourage individuals to go to **A&E** if they are worried that they are at
immediate
risk of harming themselves

3. Plan – Identify whether the individual has a plan for how they might act on these thoughts

4. Means – Identify whether the individual are in a safe space at the moment?

Has means currently available to them to follow through with a plan?
(i.e.: weapon, stock of medication)

5. Immediacy – Identify whether the individual is in immediate danger of acting on this plan

If individual is expressing immediate intent, ask the person can they guarantee their safety at this present time? - **Explain to the patient that you are concerned for their safety and are going to call in a Senior Psychologist to join the call:**

If YES	If NO
- Establish a safety contract (i.e., patient agrees to stay safe for fixed period of time) - Consent to brining someone on the call (i.e., if someone is at home with them) - <u>Contact Psychologist on Call</u> - Focus on reasons of living e.g., loved ones - Contact GP for an appointment to discuss these thoughts and a possible treatment plan going forwards - Call Helplines e.g., Pieta House/ Samaritans	- Advise they go to A&E immediately - If the person refuses to go to the A&E and you are worried about their immediate risk, contact their next of kin / the Gardaí - <u>Contact Psychologist on Call</u>

1. Phone Niall/ Senior Psychologist to ascertain availability
2. Introduce patient & explain that they have disclosed information that they are in immediate danger to themselves
3. Invite Niall/ Senior Psychologist to Attend Anywhere
4. Niall / Senior Psychologist can speak to patient directly through Attend Anywhere
5. Report to GP about incident if needed for follow-up

Psychologist	Days Available	Contact Number
Niall Pender	Mon	01 809 2414 / 086 8161241
Lauren's (Secretary) Office [for other available psychologist]	Tues, Weds, Thurs, Fri	01 809 2223

Contact details of Helplines:

Pieta House: **1800 247 247**

Text 50808: text **HELLO** to **50808**

Samaritans: **166 123** - email: jo@samaritans.ie

GP and health centres

A GP can offer support for anyone in crisis. If possible, ask someone to come go with them.

Find a service: [GP or health centre](#) / [Out of hours GP service](#)

Appendix 3 Online study protocols (Chapter 3)

HD-Cognition online recruitment protocol (Version 1.2)

Stage 1: Initial contact

- 1.1 Phone contact from database, explain who we are and why being contacted. Be mindful of notes and any previous contact. Ask if they would be interested in participating and if yes, give brief summary of the study and what is involved.
Key points
 - 2.5 hour cognitive assessment (thinking tests) and brief interview about family history etc online;
 - Online questionnaires to be done in own time at home (total of about 20 mins);
 - Repeated in 12-18 months.
- 1.2 If interested, get their email address, and confirm it, and other contact information if not on the database – want to have everything on there, i.e.: DOB, home address, mobile number, email address etc.
- 1.3 Also confirm their genetic status on the phone call, if known, and which group they would be in
- 1.4 Tell them you will be sending an email with study info and a link to the consent form which includes a brief demographic questionnaire [1.1 Full Consent and Demographics - ...]. This participant information leaflet (PIL) will contain information on Huntington's disease and the study.
- 1.5 Confirm that this is ok and the email address they provided is a good one to receive it.
- 1.6 Emphasise taking time to read the PIL and encourage them to ask any questions before completing the consent form and demographic questionnaire. Tell them that once happy, can click the link to complete the consent form and brief questionnaire.
- 1.7 When they have completed that, or within two weeks, we'll be in touch to book the virtual assessment for a time that suits
- 1.8 Send brief email with Email Template 1.1, including PIL and link to consent form
- 1.9 Make note in database of date that they need to be followed up [one week].

Stage 2: (Phone call after 1 week)

- 2.1. Ask if all was ok with the consent form and questionnaire, and if they have any questions.
 - a. If they haven't completed the consent form:
 - i. prompt them to do so and ask if they have any questions about the study info leaflet.
 - ii. say we will be in touch in a week to follow up and book an appointment when they have completed this
 - iii. make note in database of date that they need to be followed up [one week].
- 2.2. When consent form and demographic questionnaire completed. Book in appointment for approx. 2 weeks in advance
- 2.3. Tell them you will be sending online questionnaires to complete in advance and remind them of what is involved in the assessment. Remind them that these are online questionnaires about their thoughts, feelings and behaviour

- 2.4. **NB:** Explain about **unique participant ID** and the importance of entering this correctly in the questionnaire.
- 2.5. **NB:** Questionnaire should only be completed within 1 week of assessment. Send in reminder email at 1 week if full battery is scheduled at later date in the future post consent.
- 2.6. Send email confirming appointment, with info leaflet about getting computer and environment set up and word document (lowercase surname, no punctuation) attached. This is Email Template 2, which includes link to platform (Attend Anywhere: <https://attenduk.vc/bmclinicalservices>), and link to questionnaire [3. HD-Cog Questionnaires - ...] with **unique participant ID code** to enter into the survey (emphasise this in phone call). Encourage to ask questions about questionnaires if needs be.
- 2.7. Tell about need for some blank sheets of paper and a dark pen / marker for the assessment (RCFT).
- 2.8. Call to remind about appointment 2 days in advance and resend appointment email if necessary.
- 2.9. Ensure minimum technology requirements:
 - a. tablet or laptop screen larger than 9”;
 - b. high speed Wi-Fi.

Stage 3: Prior to Neuropsych assessment:

- 3.1. Download from Qualtrics and print off consent form and demographics
- 3.2. Take consent form and any sheets with identifiable information (e.g.: Name, Address) and add them to a ‘Consent form’ folder, where it will be stored in the locked filing cabinet drawer.
- 3.3. Review questionnaires for risk [see risk protocol], and highlight any questions in the demographics or study questionnaires that need to be followed up with / clarified in the interview.
- 3.4. Confirm phone number of participant and next of kin.

Stage 4: Neuropsych assessment:

- 4.1. Do interview at start of neuropsych assessment. If completed, ask explicitly about online questionnaires and if any questions / difficult emotions were brought up by the questionnaires / process (i.e.: risk protocol), they can be addressed here. [If not completed, 4.1.1]
- 4.2. As part of interview, clarify details and expand on past medical history and Family History questions to get full family history, including onset symptoms and age of parents / relatives with HD and psychiatric family history.
- 4.3. At end, thank for participation and remind about annual follow up.
- 4.4. Ask if a companion / caregiver could answer a few questions about them for the research. If yes, get their email / phone number.
- 4.5. Ask if there are other family members who would be interested in participating. If yes, ask for phone number / email to contact. If not willing, send information leaflet for other family member to be sent around.

Stage 4: (if questionnaires not completed)

- 4.1.1. Remind in assessment that they should complete the questionnaires as soon as they can if they haven't already done so and that we'll ring to check in in a week if they are having any trouble with them. Can resend link if needs be
- 4.1.2. Add date in database for follow up in one week
- 4.1.3. At follow up, if haven't completed questionnaires, prompt one more time to do so. Failing completion this time, can be noted as incomplete
- 4.1.4. At follow up, if questionnaires have been completed, check for risk etc. Ask explicitly about questionnaires and if any questions / difficult emotions were brought up by the questionnaires / process. Thank for participation and remind about annual follow up.

Online study protocol shown to and discussed with participants at the start of each study appointment.

NOTE: Same screen shown to HD-Cognition participants with study name changed.

- This assessment is being completed as part of your participation in a research study entitled "*A study of Multiple Effects of Genes Associated with MND*" – The ALS Family Study
- This research is conducted by Professor Orla Hardiman, Consultant Neurologist in Beaumont Hospital and Professor Niall Pender, Principal Clinical Neuropsychologist in Beaumont Hospital. It is funded by Research Motor Neurone.
- I have obtained consent from you to perform this assessment.
- Study procedures have changed to adapt with the COVID-19 situation. No changes apply to the type of information that is collected or how the data will be treated; the only difference remains in the means used to collect the data.
- Even though we are using a secure system, fully compliant with data protection and protected health information (PHI) restrictions, there cannot be 100% guarantee of security when using online technology.
- The examiner is within the hospital premises, in a private room where there is no risk of being overheard.
- The assessment will take approximately 2.5 hours to complete and you are encouraged to take breaks if needed.
- No aspect of this assessment is being recorded, and we would also ask that you do not record the session as doing so could interfere with the outcomes from this research.
- Do not write down any of the answers unless otherwise instructed.
- You will be provided with instructions as we proceed through the assessment. For some tasks, I will be sharing the screen.



AttendAnywhere videoconferencing software information leaflet sent via email to participants with confirmation of study appointment:

Attending your appointment by video



Beaumont Clinical Services

Video calling is convenient and easy to use

Instead of travelling to your appointment, you will enter the online waiting area, using the link below.

The service will see that you have arrived, and a provider will join your call when ready.

There is no need to create an account.

Video calls are secure and your privacy is protected.



How do I attend my video appointment?

Go to: <https://attenduk.vc/bmclinicalservices>

What do I need to make a video call?

- ✓ **A device** for making a video call, such as a smartphone, tablet, or computer with a webcam and speaker (often built into laptops).
- ✓ **A reliable internet connection** (wired, WiFi or mobile data). If you can watch a video online, you should be able to make a video call.
- ✓ **A private, well-lit area** for your consultation, where you will not be disturbed.

✓ Use the latest version of **Chrome, Safari,** or **Edge** for best video calling performance.



Google Chrome
Windows, Android,
MacOS



Apple Safari
MacOS, iOS,
iPadOS



Microsoft Edge
Windows

See page two for more information on how to make a video call

What do I need to know?

Is it secure / confidential?

You have your own private video room that only authorised providers can enter.

No information you enter is stored.

Calls are not recorded.

How much does a video call cost?

The video call is free except for your internet usage.

Tip! If you can, connect to a Wi-Fi network to avoid using your mobile data.

How much internet data will I use?

The video call doesn't use data while you are waiting for someone to join you.

After the call connects, it uses a similar amount of data to Skype® or FaceTime®.

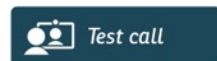
How do I make my video call?

Open your web browser and type the service's web address into the address bar (not the search box).

When you're ready, click the **Start video call** button.



Tip! Make a test call before your appointment to check your setup.



The system will check that you have everything in place (internet, speaker, microphone, and video).



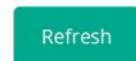
If asked, allow your browser to access your camera and microphone.

Enter your details, tick the box to agree to the *Terms and Conditions*, and start your call.



To end the call, click **End**.

Tip! Many call issues can be fixed by clicking **Refresh**.



What do I do if something's not working?

Visit <https://consult.attendanywhere.co.uk/troubleshooting>

More information

<https://www2.hse.ie/services/attend-anywhere/attend-anywhere.html>

Call Colm: 089 4123308



See page one for your service's web address

Appendix 4 End of Qualtrics questionnaire information (Chapter 3)

End of Survey

English (UK) ▾

Thank you for completing this survey, your answers have been collected. Your insights are key to helping us understand more about HD, and so we really appreciate you taking the time to help.

Some of the questions you have just answered addressed sensitive topics that you may have had some personal experience with and which you may have found distressing. If you have been negatively impacted by engaging with these topics today please get in touch with the appropriate support services who will be able to assist you – we have listed some helpful contacts below:

Samaritans

The Samaritans telephone service is available 24 hours a day.

For confidential, non-judgmental support:

Freephone: 116 123

Email: jo@samaritans.ie

Visit www.samaritans.ie for details of the nearest branch

Text 50808 is a free 24/7 text service, providing everything from a calming chat to immediate support for people going through a mental health or emotional crisis - big or small.

Text **HELLO** to **50808** to start a conversation, any time - day or night.

Visit www.text50808.ie for more information.

GP and health centres

If you have any concerns about your mental health or the mental health of a family member, please contact your GP. A GP can offer support for anyone in crisis. If possible, ask someone to come along with you.

Find a service near you:

[GP or health centre](#)

[Out of hours GP service](#)

If you have any questions regarding Huntington's Disease you can contact the Huntington's Disease Association of Ireland (HDAI) on:

Tel: 01 872 1303

Helpline: 1800 393939

Email: info@huntingtons.ie

Website: www.huntingtons.ie

If you have any queries regarding this research, please see the **Information Sheet** provided to you in the email, or you can contact the researchers using the information below:

Colm Peelo

Dept. of Neuropsychology, Beaumont Hospital

Email: cpeelo@tcd.ie

Appendix 5 Dimensional Apathy Scale Clinical cut-off scores.

From Radakovic et al. (2016) (Chapter 3)

Appendix 5 Table: *Dimensional Apathy Scale cut-off scores for abnormality based on normative data in Radakovic et al.2016.*

	Abnormality cut-off
DAS Total	39
DAS Executive domain	14
DAS Emotional domain	15
DAS Initiation domain	16

Maximum score for each domain is 24 and Total is 72.

Appendix 6 ECAS abnormality cut-off scores (Chapter 3)

Normative data and clinical abnormality-cut-off scores for ECAS A stratified for age and years of education from Pinto-Grau et al., 2017.

Age	<65				≥65			
Years of education	<12		≥12		<12		≥12	
	(n = 18)		(n = 26)		(n = 17)		(n = 21)	
	Mean (SD)	Abnormality Cut-off	Mean (SD)	Abnormality Cut-off	Mean (SD)	Abnormality Cut-off	Mean (SD)	Abnormality Cut-off
ECAS Total Score	114.5 (8.99)	96	118.38 (8.77)	100	108.76 (15.93)	76	116.62 (10.54)	95
ALS-Specific	84.78 (7.06)	70	87.73 (7.3)	73	81.18 (11.79)	57	85.48 (8.63)	68
ALS Non-Specific	29.72 (3.12)	23	30.65 (3.16)	24	27.59 (5.97)	15	31.14 (3.07)	25
Language	26.72 (1.53)	23	27.46 (0.81)	25	25.12 (3.48)	18	27.29 (1.31)	24
Verbal Fluency	19.11 (2.59)	13	19.85 (2.19)	15	18.94 (2.36)	14	20.38 (1.75)	16
Executive	38.94 (4.93)	29	40.42 (5.73)	28	37.12 (7.66)	21	37.81 (6.9)	24
Memory	18.06 (3.26)	11	18.81 (2.97)	12	16.24 (5.12)	6	19.29 (2.83)	13
Visuospatial	11.67 (0.49)	10	11.85 (0.46)	10	11.35 (1.06)	9	11.86 (0.65)	10

Appendix 7 ECAS Verbal fluency index conversion scores (Chapter 3)

Verbal fluency index (VFI) conversion scores for ECAS A (free fluency = letter 'S'; restricted fluency = letter 'T') from Pinto-Grau et al., 2017.

VFI conversion to score – Letter S			VFI conversion to score – Letter T		
Spoken VFI	Written VFI	Score	Spoken VFI	Written VFI	Score
≥15.68	≥25.86	0	≥34.56	≥52.35	0
12.88 to <15.68	20.92 to <25.86	2	28.23 to <34.56	42.93 to <52.35	2
10.07 to <12.88	15.99 to <20.92	4	21.90 to <28.23	33.51 to <42.93	4
7.27 to <10.07	11.05 to <15.99	6	15.57 to <21.90	24.09 to <33.51	6
4.46 to <7.27	6.12 to <11.05	8	9.24 to <15.57	14.67 to <24.09	8
1.66 to <4.46	1.18 to <6.12	10	2.91 to <9.24	5.25 to <14.67	10
<1.66	<1.18	12	<2.91	<5.25	12

Verbal fluency index (VFI) conversion scores for ECAS B (free fluency = letter 'F'; restricted fluency = letter 'D') and ECAS C (free fluency = letter 'P'; restricted fluency = letter 'M'), from Costello et al. 2020.

ECAS B				ECAS C				Converted Score
Spoken		Written		Spoken		Written		
Unrestricted	Restricted	Unrestricted	Restricted	Unrestricted	Restricted	Unrestricted	Restricted	
≥ 14.9	≥ 25.6	≥ 19.1	≥ 37.2	≥ 13.3	≥ 25.3	≥ 13.1	≥ 31.1	0
13.2 to 14.9	21.3 to 25.6	15.9 to 19.1	30.8 to 37.2	11.2 to 13.3	20.9 to 25.3	10.9 to 13.1	25.7 to 31.1	2
10.5 to 13.2	17.0 to 21.3	12.7 to 15.9	24.5 to 30.8	9.1 to 11.2	16.6 to 20.9	8.7 to 10.9	20.4 to 25.7	4
7.9 to 10.5	12.7 to 17.0	9.4 to 12.6	18.2 to 24.5	7.0 to 9.1	12.2 to 16.6	6.5 to 8.7	15.1 to 20.4	6
5.3 to 7.9	8.4 to 12.7	6.2 to 9.4	11.8 to 18.2	4.9 to 7.0	7.8 to 12.2	4.2 to 6.5	9.8 to 15.1	8
2.6 to 5.3	4.1 to 8.4	3.0 to 6.2	5.5 to 11.8	2.8 to 4.9	3.4 to 7.7	2.1 to 4.2	4.4 to 9.8	10
< 2.6	< 4.12	< 3.0	< 5.5	< 2.8	< 3.4	< 2.1	< 4.4	12

Appendix 8 ECAS Reliable change indices (Chapter 3)

Reliable change index (RCI) scores for ECAS A, B, and C from Costello et al., 2020.

	ECAS total	ALS specific	ALS nonspecific
ECAS A–B	–9.57 to 16.91	–8.03 to 13.79	–4.35 to 5.69
ECAS B–C	–7.45 to 6.67	–7.93 to 5.29	–2.64 to 4.6
ECAS A–C	–5.66 to 11.82	–6.51 to 9.07	–2.66 to 5.96

Appendix 9 UK Biobank Thoughts And Feelings Questionnaire (Chapter 3)

Neuropsychiatric Questionnaire	Psychiatric Trait / Behaviour Assessed
UK Biobank Thoughts and Feelings Questionnaire	Broad self-reported mental health and cognitive-affective functioning
Patient Health Questionnaire-9 (PHQ-9)	Presence and severity of depression
Generalised Anxiety Disorder – 7 (GAD-7)	Presence and severity of anxiety
Composite International Diagnostic Interview Short Form (CIDI-SF)	Lifetime incidence of depression, anxiety, panic attacks, and OCD based on DSM-IV criteria
Psychosis Screening Questionnaire (PSQ)	Presence and severity of positive symptoms of psychosis and schizophrenia
Alcohol Use Disorders Identification Test (AUDIT)	Presence and severity of excessive alcohol consumption
Obsessive-Compulsive Inventory – Revised (OCI-R)	Obsessions and compulsions, including washing, checking, ordering, obsessing, hoarding, and neutralising
Barrett Impulsiveness Scale (BIS-11)	Impulsivity across five domains: attention, cognitive stability, perseverance, self-control, cognitive complexity
Dimensional Apathy Scale (DAS)	Apathy in three different dimensions; executive, emotional, and cognitive/behavioural initiation
Autism Spectrum Quotient (AQ)	Social and non-social behavioural and cognitive traits associated with autism
Adult ADHD Self-Report Scale (ASRS)	Symptoms of ADHD/ADD based on DSM-IV criteria
Community Assessment of Psychic Experiences (CAPE-P15)	Positive psychotic symptoms including persecutory ideation, bizarre experiences, and perceptual abnormalities
The Ten Item Personality Inventory (TIPI)	Five core personality traits: extraversion, openness, agreeableness, neuroticism, and conscientiousness

From Costello (2022)

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Appendix 10 HD-Carer: Caregiver questionnaires (Chapter 3)

Questionnaires completed by informants of premanifest and manifest PwHD participants as part of the HD-Carer study, occurring alongside the HD-Cognition study.

<i>Caregiver / Family Member Questionnaires</i>	
The Modified Caregiver Strain Index (MCSI)	Short self-report questionnaire measuring caregiver strain in informal caregivers
Zarit Burden Interview (ZBI)	Self-report measure of burden in informal caregivers – 12 item short form
Coping in Stressful Situations (CISS)	Self-report measure of coping styles with three categories of coping
The Positive Negative Relationship Quality Scale (PNRQ)	Self-report measure of bidimensional relationship quality
The Post-Traumatic Growth Inventory (PTGI)	Self-report questionnaire assessing the positive outcomes occurring as a consequence of trauma, or in this case diagnosis of a life-limiting neurodegenerative disease

References:

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Appendix 11 Diagnosing ALS-Frontotemporal Spectrum Disorder using the ECAS from Gregory et al. (2020) (Chapter 5)

Diagnosing ALS-Frontotemporal Spectrum Disorder using the ECAS Abrahams 2018

ALS-FTSD has been defined in new diagnostic criteria by Strong et al. (2017). The following indicates how the ECAS can be used to aid the diagnostic process.

For diagnosing ALSci

A deficit in either ECAS Total OR ALS Specific Scores.

Justification: The recently revised diagnostic criteria for diagnosing ALS-frontotemporal spectrum disorder recommended that when ALS screening tests and brief assessments such as the ECAS are employed, published cut-offs are used to determine ALSci (Strong et al. 2017). Niven et al. (2015) demonstrated that maximum sensitivity and specificity to detect ALS specific cognitive impairment is achieved through combined use of a deficit in either the ALS-Specific or ECAS Total scores.

For diagnosing ALSbi

The presence apathy

OR the presence of two other behaviour symptoms as found in the ECAS behaviour interview (disinhibition, loss of sympathy, perseveration, change in eating behaviour, psychosis).

Loss of insight is not specifically measured by the ECAS but can be included through clinical judgement as one of the other behaviour symptoms

For diagnosing ALS-FTD

Evidence of progressive deterioration in behaviour and/or cognition by observation or history
And

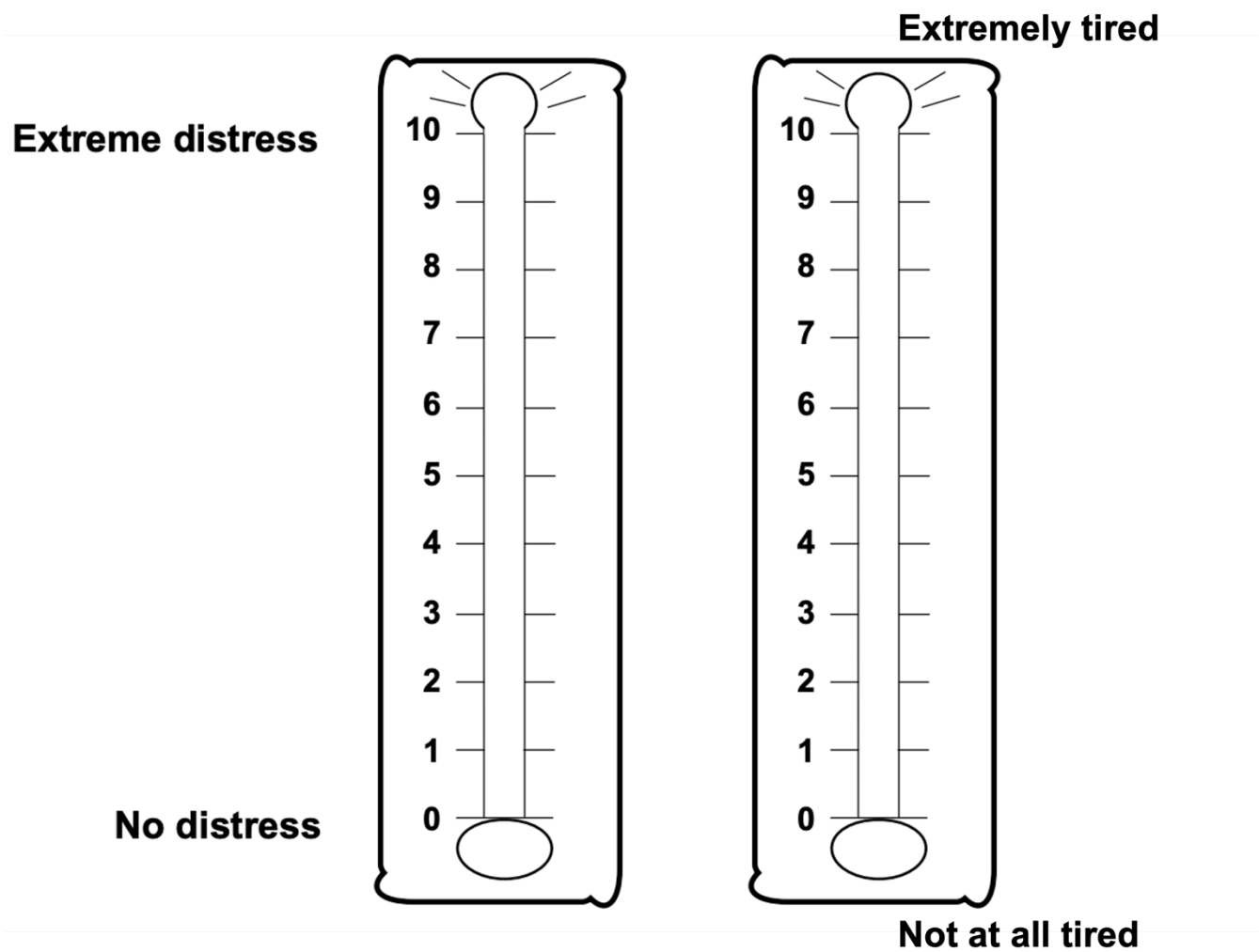
The presence of at least 2 behaviour symptoms (disinhibition, apathy, loss of sympathy, perseveration, eating behaviour) in addition to a deficit in ALS-Specific ECAS Score,
OR the presence of at least 2 behaviour symptom together with psychotic symptoms and/or loss of insight.

Diagnosis should be made by an experienced clinician or discussed within the MDT. It should be noted that in accord with the diagnosis of behavioural variant FTD diagnosis can be made without the presence of cognitive impairment.

Strong et al 2017 have an additional criteria which is the presence of language impairment meeting criteria for Semantic Dementia or Non-Fluent progressive aphasia (e.g. Gorno-Tempini et al. 2011). These conditions are rare (Saxon et al. 2018), diagnosis is complex particularly if the patient has bulbar dysfunction and should be undertaken by a neuropsychologist. The ECAS can be used to aid diagnosis together with other neuropsychological tests.

Appendix 12 Tiredness and Distress Thermometer (Chapter 5)

Adapted from Ownby et al. (2019)



Supplemental information

Appendix 13 Model output and descriptive statistics for distress and cognition in the MND clinic study (Chapter 5.2)

Multiple regression model for raw distress score for all predictors except behavioural scores. Outcome variable = DT Score.

Distress model	β (SE)	t	p
Constant	6.673 (2.999)	2.226	.031*
Age	-0.051 (0.041)	-1.246	.219
Sex (Female)	0.751 (0.848)	0.886	.381
Duration since dx	-0.006 (0.008)	-0.785	.437
Wait time	0.006 (0.006)	0.988	.328
Distance Travelled	-0.001 (0.005)	-0.207	.837
Tiredness rating	0.25 (0.141)	1.774	.083.

F(5,45)=1.27, p=.3; adj. R²=0.026.

*: significant at the 0.05 level. .: Approaches significance.

Mean and standard deviations for scores on the Tiredness thermometer, and other clinical variables in the Distress and cognition in the MND clinic study.

	Whole group	High Distress	Low Distress
n	60	33 (55%)	27 (45%)
Tiredness ratings	3.82 (3.03)	4.64 (3.06)	2.85 (2.73)
n	56	31	25
Wait times	170.5 (73.24)	182.16 (78.56)	156.04 (64.68)
n	60	33	27
Distance travelled	111.37 (84.72)	112.0 (86.16)	110.59 (84.56)
n	56	32	24
Diagnostic latency (months)	13.54 (10.49)	15.0 (12.57)	11.58 (6.57)

Appendix 14 Evaluating Attrition bias in the longitudinal MND Family Study (Chapter 6)

Table below gives baseline demographic data and descriptive statistics for those who declined participation in Phase 2. Welch's two-sample t-test showed participants who declined Phase 2 had significantly lower education ($t(81.1) = -2.32$, $p = .023$) and trended towards being older ($W = 1182$, $p = .16$). There was no difference in the proportion of *C9orf72*-carriers overall ($\chi^2(1)=0.002$, $p=.96$), although a high proportion declined extended neuropsychological assessment in Phase 1 (77.8%). Decliners had significantly lower scores on ECAS Total ($W = 762.5$, $p = .047$), and trended towards lower scores on ALS-Specific and ALS-non-Specific domains also ($p > .05$). Fisher's exact test found participants with abnormal ECAS Total scores were 4.8-times as likely to decline participation in Phase 2 ($OR=4.75$, $95\%CI:[1.17,28.11]$, $p=.025$).

Decliners who completed the extended neuropsychological assessment at baseline did not differ from completers on phonemic verbal fluency (FAS; $W = 608$, $p = .168$), working memory (DSB; $t(48.87) = -0.14$, $p = .89$) or premorbid IQ estimates (TOPF; $t(68.59) = 0.502$, $p = .617$). Decliners had significantly lower confrontation naming performance on the Boston Naming Test (BNT; $W = 314$, $p = .012$).

Attrition descriptive statistics: Baseline data for participants who completed vs declined participation in Phase 2 after baseline assessment (T1).

Completed Phase 2		Declined				p ^a
Baseline data		FB T1 only ^b	Phase 2	FB T1 & Phase 2	Decliners total	
n	42	9	26	22	57	
Age	43.26 (15.5)	38.67 (9.1)	51.96 (19.9)	46.0 (13.9)	47.56 (16.9)	.164
Sex (% female)	47.7%	20%	50.0%	72.7%	52.6%	.315
Years Education	17.46 (2.9)	15.39 (3.2)	15.85 (3.5)	15.34 (5.7)	15.58 (4.3)	.023*
C9 Positive %	24.4%	77.8%	15.4%	40.9%	27.1%	.963
C9 Family %	60%	9 (100.0%)	7 (26.9%)	100.0%	66.7%	.782
<u>Reason:</u>						
Declined	-	100.0%	26.9%	50.0%	47.4%	-
Unsuitable	-	0.0%	19.2%	0.0%	8.8%	-
No response	-	0.0%	53.8%	50.0%	43.9%	-
ECAS Total Score	111.38 (9.69)	114.44 (6.6)	104.54 (13.4)	105.82 (17.12)	106.6 (14.5)	.047*
% Abnormal	7.1%	0.0%	30.8%	22.7%	22.8%	.025*
ALS-Specific Score	82.02 (7.99)	85.11 (4.7)	76.65 (11.1)	78.18 (15.3)	78.58 (12.5)	.161
% Abnormal	11.9%	0.0%	30.8%	18.2%	21.1%	.177
ALS-non-Specific Score	29.36 (3.88)	29.33 (3.04)	27.89 (4.4)	27.64 (4.1)	28.02 (4.1)	.051.
% Abnormal	9.5%	0.0%	19.2%	4.5%	10.5%	.745
ECAS Fluency	18.55 (3.5)	18.44 (2.6)	16.46 (5.2)	17.00 (5.2)	16.98 (4.8)	.732
TOPF-FSIQ	105.53 (11.37)	104.84 (12.2)	101.55 (14.8)	-	102.83 (14.0)	.617
FAS (z-score)	-0.54 (1.04)	-0.25 (0.72)	-0.37 (1.07)	-	-	.168 ^c
DSB (z-score)	0.09 (0.97)	0.60 (1.0)	0.05 (0.95)	-	-	.890 ^c
BNT (z-score)	-0.48 (0.65)	-0.13 (0.37)	-1.38 (1.59)	-	-	.012* ^c

^a Comparing Phase 2 decliners and participants who completed both phases. For continuous variables, t-tests were employed for parametric data, Wilcoxon rank-sum test for non-parametric data. Categorical data were tested for independence using Chi-Square tests or Fisher's exact test. ^b declined FB in Phase 1, but completed T1 extended neuropsychological assessment in Phase 2 with ECAS T2. ^c comparison of completers and those who completed the extended neuropsychological assessment at T1 but not Phase 2.

Total sample recruitment numbers by assessment completed.

	Total sample			
	ECAS		FB	
	T1	T2	T1	T2
Control	186	31 ^a	97	34
Relatives	273	52	216	42

^a n = 12 excluded from longitudinal ECAS analysis as completed ECAS B at T1.

NOTE: all T2 relatives are fALS.

Appendix 15 MND Family Study: Phase 2 Longitudinal follow up (Chapter 6)

Longitudinal follow-up scores for extended neuropsychological assessment examining endophenotype stability in fALS relatives.

	T1		T2		T2-T1 ΔM (ΔSD)	
	Controls	fALS Relatives	Controls	fALS Relatives	Controls	fALS Relatives
n	32	42	32	42	-	-
TOPF-FSIQ T1 (Std. Score)	111.3 (9.13)	105.53 (11.37)	-	-	-	-
Primary tests of interest						
WASI-FSIQ-2 (Std. Score)	112.77 (9.50)	99.98 (15.73)	112.63 (12.46)	104.67 (12.5)	0.424 (10.82)	4.69 (13.64)
FAS Total	0.01 (1.19)	-0.54 (1.04)	0.31 (1.07)	-0.15 (1.09)	0.202 (0.83)	0.387 (0.9)
DS-Backward span	0.7 (1.02)	0.09 (0.97)	0.88 (1.09)	0.21 (0.9)	0.224 (0.85)	0.121 (1.17)
BNT Spontaneous	0.16 (0.73)	-0.48 (0.65)	0.11 (0.43)	-0.09 (0.47)	0.141 (0.55)	0.394 (0.5)
Category Fluency	-0.18 (0.90)	0.02 (0.79)	0.64 (1.15)	-0.11 (1.07)	1.061 (1.02)	0.185 (1.1)
RFI score	0.17 (1.23)	-0.56 (1.18)	-0.57 (1.4)	-0.36 (1.33)	-0.901 (1.35)	0.201 (1.25)
Other tests						
RMET	0.15 (0.91)	0.09 (0.92)	0.27 (0.78)	0.18 (0.97)	0.114 (0.73)	0.093 (0.75)
Immediate story memory	0.73 (0.65)	0.61 (0.82)	0.7 (0.75)	0.41 (0.72)	-0.03 (0.61)	-0.2 (0.71)
Delayed story memory	0.84 (0.87)	0.42 (0.72)	0.76 (0.96)	0.52 (0.67)	-0.074 (0.78)	0.1 (0.76)
RAVLT Total	1.19 (1.1)	0.66 (1.24)	1.09 (1.1)	0.5 (0.96)	-0.197 (1.11)	-0.157 (0.88)
RAVLT Delayed recall	0.82 (0.9)	0.2 (1.05)	0.75 (1.09)	0.24 (0.91)	-0.066 (0.68)	0.04 (0.78)
RAVLT recognition	0.61 (0.52)	0.53 (0.65)	0.87 (0.43)	0.68 (0.57)	0.214 (0.46)	0.14 (0.65)
RCFT immediate	1.11 (1.64)	0.1 (1.57)	0.82 (1.13)	0.89 (1.09)	-0.289 (1.89)	0.782 (0.96)
RCFT delayed	0.56 (1.22)	0.44 (3.69)	0.82 (1.28)	0.79 (1.2)	0.261 (0.94)	0.868 (1.1)
DS-Forward span	0.67 (1.01)	0.38 (0.97)	0.47 (0.99)	0.13 (0.89)	-0.192 (1.09)	-0.247 (0.91)
CWIT inhibition errors	0.4 (0.54)	0.3 (0.49)	0.35 (0.53)	0.42 (0.48)	-0.052 (0.72)	0.116 (0.57)
CWIT inhibition time	0.5 (0.61)	0.12 (0.77)	0.64 (0.64)	0.49 (0.68)	0.141 (0.41)	0.389 (0.77)

	T1		T2		T2-T1 ΔM (ΔSD)	
	Controls	fALS Relatives	Controls	fALS Relatives	Controls	fALS Relatives
CWIT switching errors	0.43 (0.49)	0.23 (0.5)	0.44 (0.52)	0.25 (0.55)	0.022 (0.64)	0.019 (0.64)
CWIT switching time	0.37 (1.03)	0.21 (0.85)	0.54 (0.82)	0.32 (0.79)	0.183 (0.8)	0.12 (0.71)
HADS anxiety (raw score)	4.5 (2.42)	4.69 (3.8)	6.53 (4.58)	4.26 (3.25)	-0.455 (2.77)	-0.485 (2.24)
HADS depression (raw score)	1.38 (1.31)	1.8 (2.24)	3.73 (3.63)	1.4 (1.52)	0.909 (3.42)	-0.455 (2.0)
GHQ (raw score)	10.64 (2.38)	10.34 (4.83)	8.5 (2.39)	9.34 (2.94)	-2.375 (2.92)	-0.938 (4.26)

NOTE: Scores are given as Mean (SD) and z-scored based on published normative data controlling for education and/or age, unless otherwise stated. T2-T1 ΔM (ΔSD)=Mean (SD) of change scores

Cumulative distribution of RFI z-scores in each phase by group.

n (%)	$z \leq -3$	$z \leq -2$	$z \leq -1$	$z \leq 0$	$z \leq 1$	$z \leq 2$	$z \leq 3$	$z \leq 4$
Phase 1								
Control	0 (0%)	0 (0%)	7 (13%)	24 (44.4%)	39 (72.2%)	48 (88.9%)	52 (96.3%)	54 (100%)
Relative	0 (0%)	5 (5.4%)	23 (25%)	62 (67.4%)	81 (88%)	90 (97.8%)	92 (100%)	92 (100%)
Phase 2								
Control	1 (2.8%)	1 (2.8%)	5 (13.9%)	17 (47.2%)	31 (86.1%)	36 (100%)	36 (100%)	36 (100%)
Relative	3 (4.6%)	9 (13.6%)	31 (47%)	46 (69.7%)	57 (86.4%)	64 (97%)	65 (98.5%)	66 (100%)

NOTE: Excluding cases missing FAS or Semantic fluency scores and therefore RFI data.

RFI Score distribution

Cumulative distribution of RFI values across the two timepoints can be seen in table below. Controls follow an approximately normal distribution at baseline (T1). 71% of fALS relatives have a $z \leq 0$, compared to 44.8% of controls. Follow-up T2 RFI scores do not follow a similar distribution for controls, with much higher proportions falling below $z = -1$ (37.5%). Control proportions increase at the lower end of z . Relatives' proportions remain the same or decrease slightly so that they show roughly the same proportions in each bin.

Cumulative distribution of RFI z-scores in each timepoint by group.

n (%)	$z \leq -3$	$z \leq -2$	$z \leq -1$	$z \leq 0$	$z \leq 1$	$z \leq 2$	$z \leq 3$	$z \leq 4$
Baseline (T1)								
Control	0 (0%)	0 (0%)	5 (17.2%)	13 (44.8%)	23 (79.3%)	28 (96.6%)	28 (96.6%)	29 (100%)
Relative	0 (0%)	4 (9.5%)	14 (33.3%)	30 (71.4%)	36 (85.7%)	42 (100%)	42 (100%)	42 (100%)
Follow-up (T2)								
Control	2 (6.3%)	5 (15.6%)	12 (37.5%)	20 (62.5%)	27 (84.4%)	32 (100%)	32 (100%)	32 (100%)
Relative	2 (4.8%)	4 (9.5%)	13 (31%)	24 (57.1%)	36 (85.7%)	41 (97.6%)	42 (100%)	42 (100%)

NOTE: Excluding cases missing FAS or Semantic fluency scores and therefore RFI data.

Appendix 17 TOST upper and lower bounds creation for equivalence over time (Chapter 6)

Ranges and descriptives of change scores for controls and relatives on the extended neuropsychological tests over two timepoints. Scores are z-scores unless otherwise stated.

Controls (used to create upper/lower bounds)	$\Delta T2-T1$ Range	Median Δ	Mean Δ
FAS	-2.179,1.696	0.165	0.202
DSB	-1.539,1.790	0.251	0.224
BNT	-0.952,1.429	0.00	0.141
Semantic Fluency	-1.23,4.273	0.761	1.061
WASI-II FSIQ (Standard score)	-21,27	1.00	0.424
RFI	-3.29, 2.32	-1.02	-0.90
Unaffected fALS Relatives	$\Delta T2-T1$ Range	Median Δ	Mean Δ
FAS	-0.982,3.304	0.266	0.387
DSB	-2.667,3.467	0.028	0.120
BNT	-0.735,2.145	0.355	0.394
Semantic Fluency	-3.519,2.421	0.185	0.185
WASI-II FSIQ (Standard score)	-25,40	4.50	4.69
RFI	-2.75, 3.84	0.021	0.201

NOTE: Control change scores were used to calculate the lower and upper bounds for the TOST equivalency test ($\Delta M \pm 0.5(SD)$).

Appendix 18 Longitudinal MND Family Study: ECAS *C9orf72*

subgroup analyses (Chapter 6)

ECAS domain abnormality classifications for fALS relatives by C9orf72-status.

	T1		T2		RCI Decline
	C9-	C9+	C9-	C9+	n
ECAS Total	4 (11.8%)	0 (0.0%)	2 (5.9%)	1 (6.2%)	1 ^a
ECAS ALS-Specific	6 (17.6%)	0 (0.0%)	2 (5.9%)	1 (6.2%)	2 ^a
ECAS ALS-Non-Specific	3 (8.8%)	1 (6.2%)	1 (2.9%)	1 (6.2%)	2 ^b

NOTE: n=4 individual relative cases declined significantly by RCI change scores. n=3 remained in the normal range. n=1 control was abnormal on the ALS-non-specific subtest at T1 and a different n=1 control participant had an abnormal score on the ECAS ALS-non-specific subtest at T2. No other control participants fell in the abnormal range on any other domains. RCI = Reliable Change Index. C9+/C9-=C9orf72-positive/-negative.

^a C9+ fALS relatives. n=1 declined on ALS-specific only and n=1 on ALS-specific and ECAS Total at T2.

^b n=2 C9- fALS relatives from the same family and of older age (78 & 80 years old at T2).

ECAS LMMs

‘ECAS Total & ALS-Specific’ ~ ‘Age’ + ‘Education’ + ‘C9-status*’Ax time’ + (1 | family/id)

‘ECAS ALS-non-Specific’ ~ ‘Age’ + ‘Education’ + ‘C9-status + ‘Ax time’ + (1 | family/id)

NOTE: The only difference is the absence of the interaction term of C9status and time for the ALS-non-specific ECAS domain.

Linear mixed effects model for relatives group only on the ECAS longitudinal follow up by C9orf72 status.

	B (SE)	df	t	β	p	Robust SE p ^a
ECAS Total						
Fixed effects						
Constant	91.22 (6.59)	44.92	13.847	-0.41	<.001	<.001
Age	-0.15 (0.07)	45.1	-2.102	-0.21	.041	.036
Education	1.46 (0.3)	48.18	4.944	0.47	<.001	<.001
C9 Status (C9+)	4.21 (2.5)	72.1	1.685	0.41	.096	.119
Ax Time (T1-T2)	6.95 (1.48)	53.2	4.708	0.68	<.001	<.001
Interaction (C9+ x Time)	-4.32 (2.53)	47.2	-1.709	-0.43	.094	.077
Random effects						
				SD		
ID within Family				5.13		
Family				1.85		
Residual				5.89		

Linear mixed effects model for relatives group only on the ECAS longitudinal follow up by C9orf72 status.

ECAS ALS-non-Specific	B (SE)	df	t	β	p	Robust SE p ^a
Fixed effects						
Constant	26.17 (2.59)	52.91	10.11	-0.17	<.001	<.001
Age	-0.04 (0.03)	47.91	-1.647	-0.19	.106	.043
Education	0.31 (0.12)	54.62	2.652	0.29	.011	.026
C9 Status (C9+)	-0.82 (0.83)	46.87	-0.99	-0.23	.327	.446
Ax Time (T1-T2)	1.59 (0.56)	55.76	2.843	0.45	.006	.006
Random effects				SD		
ID within Family					1.55	
Family					1.00	
Residual					2.71	

NOTE: Reported are the original unadjusted coefficients.

^a Calculated with Robust Standard errors which exclude random family effects

Appendix 19 Attrition in the HD-Cognition Study (Chapter 8)

Reasons for attrition in the main longitudinal aspect of the HD-Cognition study.

Group (at final tx)	Reason for attrition	Consented but incomplete T1	T2	T3
Manifest	Unable to contact	1	3	0
	Declined	-	1	0
	Death / Disability	-	2	0
	Assessment due	-	0	6
Premanifest	Unable to contact	3	5	1
	Declined	-	0	5
	Death / Disability / Excluded	-	0	0
	Assessment due	-	0	6
	Manifest HD ^b	-	4	2
Controls ^a	Unable to contact	16	8	3
	Declined	-	4	2
	Death / Disability	-	0	0
	Assessment due	-	0	12
Genotype Unknown	Unable to contact	2	1	1
	Declined	-	1	0
	Death / Disability	-	0	0
	Assessment due	-	13	5
Total ^b		22	38	43
DNA / cancelled appointments		8	23	15

^a Controls include patient spouses, gene negative HD family members and healthy controls.

^b Total excludes premanifest participants who moved to manifest group

Descriptive statistics for PwHD and controls who did not complete T2 in the ECAS

Validation study:

Sample descriptive statistics and demographic data for sample not completing follow-up appointment.

Decliners descriptives	Control	Premanifest	Manifest
n	13	9	5
Age	46.8 (12.2)	39.3 (8.47)	58.4 (19.2)
ECAS Total	114 (8.04)	111 (7.08)	62.4 (10.6)
TOPF	109 (13.1)	96.9 (9.2)	95.5 (12.8)
CAG available (n)	NA	8	2
CAP100	NA	89.2 (13.9)	110.0 (6.08)

Appendix 20 HD-Cognition- Longitudinal analysis (Chapter 8)

Demographic comparisons of PwHD and Control groups at T2 and T3 follow-up.

	T2					T3				
	Control	PreHD	Manifest	F/H/ χ^2 (df)	p	Control	PreHD	Manifest	F/H/ χ^2 (df)	p
n	25	29 ^a	13 ^a			9	12 ^b	10 ^b		
Age	47.56 (12.8)	46.41 (11.13)	54.23 (10.55)	2.11 (2,64)	.13	44.44 (15.8)	47.17 (11.3)	60 (9.45)	4.55 (2,28)	.02
Sex (% Female)	60%	69%	46.2%	2(2)	.4	77.8%	50%	50%	2.02(2)	.363
Years Education	17.54 (3.3)	16.67 (2.86)	15.46 (2.3)	2.18 (2,64)	.121	19.06 (2.9)	17.08 (2.99)	14.8 (2.2)	5.83 (2,28)	.008
Interval (months; Md[IQR])	18.8 [8.9]	17.1 [6.09]	18.3 [10.4]	0.1(2)	.9	18.1 [3.19]	19.7 [7.13]	22.3 [7.29]	1.36 (2,28)	.273
CAG available (n) ^b	-	26	9	-	-	-	10	6	-	-
CAG	-	41.42 (1.72)	43.56 (4)	-2(9)	.2	-	40.8 (1.23)	41.5 (1.05)	-1(12)	.2
CAG (range)	-	39-46	39-53	-	-	-	39-43	40-43	-	-
CAP100 ^{b,c}	-	83.45 (22.42)	114.32 (17.65)	-4(18)	<.001	-	81.95 (22.4)	115.42 (19.12)	-3(12)	.008
Disease Burden ^{b,d}	-	270.87 (94.53)	413.06 (105.6)	-4(13)	.003	-	252.55 (81.7)	378.08 (82.4)	-3(11)	.01
Ttdx	-	10.04 (13.56)	-6.22 (10.47)	-	-	-	11.2 (14.53)	-8 (10.97)	-	-
UHDRS-Function Md (IQR)	-	25 (0)	19 (5)	104	<.001	-	25 (0)	18 (7.25)	104	<.001
UHDRS-Function Range	-	22-25	6-24	-	-	-	25-25	12-25	-	-
UHDRS-TFC Md (IQR)	-	13 (0)	8 (5)	97	.001	-	13 (0)	8.5 (4.75)	97	.001
UHDRS-TFC Range	-	11-13	3-12	-	-	-	11-13	5-13	-	-
UHDRS-Ind. Md (IQR)	-	100 (0)	75 (20)	94	.001	-	100 (0)	85 (27.5)	94	.001

NOTE: Data presented as Mean(SD) unless otherwise stated. Ttdx=Time to predicted Diagnosis.⁷¹

a T1-T2: n=4 recategorized as manifest; b T2-T3: n=2 recategorized as manifest. UHDRS-Ind. = UHDRS-Independence scale. Md(IQR) = Median(Inter-quartile range).

Descriptive statistics for longitudinal neuropsychological assessment results in HD-Cognition study sample at T2 and T3 (**Chapter 8**).

		T2			T3		
		Controls	PreHD	Manifest	Controls	PreHD	Manifest
n		25	29 ^a	13 ^a	9	12 ^b	10 ^b
General IQ							
TOPF-FSIQ (T1)		112.13 (9.3)	103.91 (11.71)	99.71 (11.33)	115.6 (4.6)	102.33 (12.2)	102.9 (9.8)
WASI-II		114.38 (11.4)	106.54 (15)	86.6 (15.98)	121.56 (7.4)	107.92 (10.85)	91.38 (21.02)
Social Cognition							
RMET		0.61 (0.8)	0.19 (0.82)	-2.16 (0.88)	0.73 (0.6)	0.45 (0.67)	-1.49 (1.35)
ACS – Social perception battery	Affect Naming	0.43 (0.9)	-0.33 (1)	-1.69 (0.76)	0.56 (0.8)	-0.17 (1.13)	-1.4 (1.19)
	Prosody	0.57 (0.8)	0.5 (1.05)	-1.67 (0.73)	1.33 (0.8)	0.95 (0.75)	-0.44 (1.37)
	Prosody Pair total	1.24 (0.7)	0.95 (1.09)	-0.4 (0.93)	1.78 (0.6)	0.81 (0.59)	0.78 (0.84)
	Social Perception	0.62 (0.9)	0.15 (1.05)	-1.67 (0.67)	1.3 (0.9)	0.47 (0.87)	-0.39 (1.22)
Executive function							
SDMT	Total correct	-0.49 (0.9)	-1.85 (1.48)	-4.44 (1.9)	-0.4 (1)	-1.45 (1.52)	-3.92 (1.77)
	Errors (raw score)	0.5 (1.1)	0.62 (1.02)	0.92 (0.9)	0.56 (0.7)	0.92 (1)	1.5 (1.35)
Colour Word Interference Test	Naming Time	0.21 (0.8)	-0.8 (1.14)	-2.54 (1.32)	0.37 (0.6)	-0.36 (0.87)	-2.33 (1.64)
	Reading Time	0.43 (0.6)	-0.77 (1.06)	-2.56 (1.16)	0.44 (0.2)	-0.47 (0.8)	-2.07 (1.55)
	Inhibition Time	0.72 (0.7)	-0.23 (1.2)	-2 (1.33)	1 (0.5)	-0.19 (0.82)	-1.73 (1.73)
	Inhibition Errors	0.49 (0.3)	0.34 (0.54)	-1 (1.42)	0.33 (0.4)	0.42 (0.52)	-0.17 (0.83)
	Switching Time	0.7 (0.6)	-0.24 (0.83)	-1.2 (1.48)	0.96 (0.5)	-0.11 (1.2)	-0.08 (0.74)
	Switching Errors	0.43 (0.5)	0.26 (0.6)	-0.47 (1.52)	0.44 (0.3)	0.03 (1.23)	0.17 (0.8)
	Inhibition - Naming (SS)	11.54 (2.1)	11.82 (2.36)	11.25 (3.14)	11.67 (1.2)	10.5 (1.93)	11.7 (2.95)
Digit Span	Forward	0.46 (0.9)	0.06 (0.83)	-0.18 (0.99)	0.63 (0.7)	0.22 (1.03)	0 (1.21)
	Reverse	1.1 (1)	-0.08 (1.09)	-0.47 (0.87)	1.37 (0.7)	0.39 (0.75)	-0.43 (1.07)
Verbal Fluency	FAS Total	0.49 (1.1)	-0.08 (1.16)	-2.02 (1.16)	0.39 (1.1)	-0.02 (0.92)	-1.3 (1.32)
	Category (animals)	0.99 (1.3)	-0.1 (0.82)	-1.42 (1.08)	0.97 (1.1)	0 (0.82)	-0.96 (1.64)

		T2			T3		
		Controls	PreHD	Manifest	Controls	PreHD	Manifest
	RFI	-0.49 (1.4)	-0.04 (0.96)	-0.6 (0.73)	-0.58 (1.3)	-0.02 (0.99)	-0.34 (1.11)
	Restricted fluency (C; VFI)	0.32 (0.5)	0.11 (0.61)	-1.26 (1.77)	0.23 (0.4)	0.28 (0.36)	-1.05 (1.54)
Memory							
CVLT	T1-5 Total	0.6 (0.7)	0.07 (0.98)	-1.44 (0.94)	1.04 (0.6)	0.08 (0.92)	-0.67 (1.23)
	Immediate Free	0.71 (0.8)	0.1 (1.05)	-1.46 (0.97)	1.19 (0.9)	0.42 (1.01)	-0.73 (1.16)
	Immediate Cued	0.6 (0.8)	0.19 (1.17)	-1.49 (0.98)	0.89 (1)	0.22 (0.9)	-1.03 (1.41)
	Delayed Free	0.65 (0.8)	0.12 (1.19)	-1.51 (0.92)	1.22 (0.8)	0.06 (1.27)	-0.77 (1.36)
	Delayed Cued	0.4 (0.8)	0.07 (1.14)	-1.54 (0.94)	0.89 (0.7)	0.11 (0.88)	-0.87 (1.53)
	Recognition (discrim NP)	0.32 (0.8)	-0.36 (0.96)	-1.15 (0.73)	0.93 (0.6)	0.06 (1.15)	-0.57 (1.38)
	Intrusions free (raw score)	2.08 (1.6)	2.26 (2.73)	2.5 (2.75)	1.22 (1.8)	2.08 (2.28)	2.3 (2.5)
	Intrusions cued (raw score)	2.17 (2.1)	3.48 (4.27)	4.39 (1.98)	1.11 (1.5)	3.67 (5.31)	4.7 (4.42)
	Intrusions total (raw score)	7.17 (5.1)	7.37 (7.83)	9.62 (5.5)	4.33 (4.7)	9.58 (8.64)	8.8 (7.29)
	Intrusions (SS)	8.29 (2.6)	8.78 (3.4)	7.54 (2.11)	10 (3)	7.83 (3.69)	8.4 (3.17)
	Across-within intrusions (raw score)	4.21 (4.2)	4.41 (4.84)	4.54 (3.69)	2 (3)	5 (4.65)	3.7 (3.23)
	Repetition errors	6.04 (4.5)	7.19 (5.51)	3.54 (4.93)	3.11 (2.1)	6.83 (6.28)	4 (3.53)
	Logical Memory						
Logical Memory	Immediate	0.79 (0.7)	0.11 (1.01)	-1.64 (1.13)	1.19 (0.7)	0.14 (0.86)	-1 (1.26)
	Delay	0.78 (1.2)	0.31 (0.89)	-1.64 (1.1)	1.48 (0.9)	0.42 (0.83)	-0.78 (1.21)
	LM % retention	89.2 (17)	89.79 (9.76)	66.16 (27.16)	97.01 (3.8)	95.1 (6.28)	74 (21.94)
	Recognition (raw score)	25.65 (2.6)	24.79 (3.61)	19.91 (4.55)	27.89 (1.7)	24.83 (1.9)	22 (3.94)
RCFT	Copy (score)	-0.5 (1.2)	-1.46 (2.81)	-5.25 (4.91)	-0.13 (0.9)	-0.63 (1.8)	-1.84 (1.19)
	Immediate	1.06 (1.1)	0.34 (1.31)	-1.53 (1.31)	1.48 (1.1)	0.91 (1.01)	0.43 (1.79)
	Delayed	1.03 (1.1)	0.32 (1.36)	-1.53 (1.59)	1.41 (1.1)	0.92 (1.21)	0.17 (1.69)
	Recognition	0.47 (0.8)	-0.12 (1.5)	-1.84 (1.3)	0.52 (1.1)	0.59 (0.89)	0.49 (0.9)
Language							
Sydney Language	Naming	0.83 (0.5)	0.1 (1.14)	-1.68 (1.89)	0.98 (0.5)	0.31 (1.11)	-1.29 (1.9)
Battery	Comprehension	0.48 (0.6)	-0.06 (1.08)	-1 (1.74)	0.62 (0.4)	0.07 (1.06)	-2.25 (1.61)

		T2			T3		
		Controls	PreHD	Manifest	Controls	PreHD	Manifest
Cognitive Reserve (CRIq)	Semantic	0.54 (1.1)	0.13 (1.49)	-2.03 (2.12)	0.77 (0.5)	0.1 (1.58)	-3.27 (2.57)
	CRIq Total (T1)	112.23 (10.5)	112.72 (11.03)	110 (12.53)	112.25 (11)	117.5 (12.03)	112.57 (12.39)
Questionnaires (n)	Raw scores	24	22	11	8	12	7
Mood	PHQ-9	4.54 (4.2)	7.09 (6.73)	8.82 (6.84)	3.13 (2.5)	5.33 (6.36)	8.29 (8.83)
Mood	GAD-7	4 (4.6)	6.09 (5.38)	6.27 (5.29)	3 (5.4)	5.5 (5.7)	5.29 (5.62)
Disability	WHODAS Total	46.04 (11.3)	57.68 (23.17)	67.18 (28.58)	40.75 (6.8)	52.83 (20.15)	63.33 (24.12)
Quality of life	EuroQoL 6_1	81.26 (10.6)	72.59 (17.88)	72.18 (12.91)	81.78 (16.2)	73.75 (16.38)	74.14 (16.13)
Companion questionnaires (n); Raw scores		16	8	12	7	6	7
	BBI pre / in last 10 years	2.88 (4.5)	3.75 (4.95)	16.83 (15.99)	6.29 (8.6)	2 (2.28)	10 (4.32)
	BBI post / in last 3 years	3.5 (5.8)	9.13 (8.04)	35.58 (21.89)	7.57 (11.9)	6.83 (8.86)	31.86 (23.82)

^a T1-T2: n=4 recategorized as manifest; ^b T2-T3: n=2 recategorized as manifest.

RCFT = Rey Complex Figure Task. SS = Scaled Score. TOPF-FSIQ (T1) = Baseline TOPF-FSIQ. T1-5 total = Trial 1 – 5 Total score.

Data are presented as Mean(SD) of z-scores, unless otherwise stated.

Appendix 21 Rating apathy: HD-Cognition (Chapter 9)

Study 2: HD-Cognition study sample for DAS study, Chapter 9

	Controls	Premanifest	Manifest
n	33	28	16
Age M(SD)	45.5 (12.8)	47.4 (11.1)	50.9 (10.6)
Sex (% Female)	60.6	65.4	37.5
Years Education M(SD)	17.6 (2.9)	16.7 (2.9)	15.7 (2.7)
CAG available (n) ^b	-	24	10
CAG M(SD)	-	41.1 (1.5)	44.2 (3.9)
CAG (range)	-	39 – 45	39 – 53
CAP100 ^{b,c}	-	84.88 (22.8)	109.14 (17.0)
Disease Burden ^{b,d}	-	269.5 (90.5)	407.65 (98.5)
UHRS-Function Md (IQR)	-	25 (0)	22 (6.25)
UHRS-Function Range	-	22 - 25	14 - 25
UHRS-TFC Md (IQR)	-	13 (0)	10.5 (6)
UHRS-TFC Range	-	11 - 13	5 - 13
UHRS-Independence Md (IQR)	-	100 (0)	90 (21.2)
Longitudinal DAS cohort			
	Controls	PwHD	
n	20	24	
Interval (months) M(SD)	18.6 (5.8)	18.3 (4.2)	
Age M(SD)	45.0 (13.1)	49.8 (12.1)	
Sex (% Female)	60%	58.3%	
Premanifest at T1 %	-	75%	
Converters at T2 (n)	-	3	
CAG repeats	-	41.6 (3.1)	
		Premanifest	Manifest
UHRS-Function Md (IQR)	-	25 (0)	21 (4.3)
UHRS-Function Range	-	22-25	14-25
UHRS-TFC Md (IQR)	-	13 (0)	10 (4.5)

^a Not significant in post-hoc tests or after correction for multiple comparisons. ^b 6 Manifest and 2 Premanifest HD cases did not have CAG repeat numbers available to calculate Disease burden and CAP100 scores; ^c A CAP100 score of 100 is the normalised CAGxAGE product estimated age of motor symptom disease onset.³¹⁵; ^d Disease Burden calculated according to Penney et al. (1997).³¹²

Appendix 22 Rating apathy: pwALS (Chapter 9)

Study 1: pwALS full patient sample characteristics and data availability.

n		215
Age		64.86 (10.6)
Sex (% Female)		41.9%
Years Education		14.35 (3.5)
Familial ALS (by family history; %) ^a		7.9%
C9orf72 Positive (%) ^b		19.1%
Strong Criteria Categorisation ^c	ALSn	69 (34.6%)
	ALSci	20 (10.1%)
	ALSbi	81 (40.7%)
	ALScbi	29 (14.6%)
	NA ^f	16
Age at onset		63.09 (10.7)
Diagnostic latency (months)		12.99 (11.0)
Duration since diagnosis (days)		97.62 (136.8)
El Escorial Category (%)	Definite	51.7%
	Lab-supported probable	8.2%
	Probable	24.5%
	Possible	13.6%
	Suspected	2.0%
ALSFRS-R ^d		38.67 (5.9)
Onset Site (%)	Spinal	58.6%
	Bulbar	27.4%
	Thoracic / Respiratory	2.3%
	Cognitive / Behavioural	1.9%
	Unknown	9.8%
Baseline carer-rated BBI / DAS (n) ^e		183
BBI / DAS timepoint at baseline	T1	158
	T2	20
	T3	5
	Spouse	64.4%
Informant Relation (BBI / DAS rater)	Child	24.9%
	Sibling	7.3%
	Other	3.4%
Self- & Carer-rated DAS for IRR (n)		38
Complete DAS & ECAS (n)		153
ECAS Mode of admin.	% verbal	87.6%
ECAS / Tx at baseline (n)	A / T1	171

^a n=24 missing data. ^b n=121 missing data. ^c Strong Criteria Categorisations were made based on the ECAS, BBI and DAS, they do not reflect full categorisation according to Strong Criteria (2017); ^d n=62 missing data. ^e *Baseline assessment refers to all cases completing the DAS / BBI for the first time, regardless of ECAS / BBI timepoint*; ^f NA for cases without complete ECAS and behavioural data and not ALSc/bi; ALSn=ALS without cognitive or behavioural impairment. ALSFRS-R = ALS-Functional Rating Scale-revised. Data presented as *M(SD)* unless otherwise stated.

Appendix 23 Rating apathy: pwALS by genotype (Chapter 9)

Cognitive and behavioural data from pwALS sub-sample by C9orf72-genotype.

	C9+	C9-	p.adj
n	12	59	
Age	64.08 (8.49)	59.17 (11.04)	.10
Sex (% Female)	83.3%	61%	ns
Years Education M(SD)	14.63 (3.87)	14.75 (3.77)	ns
ALSFRS-R	38.55 (7.03)	38.81 (6.34)	ns
Onset site: % Bulbar	41.7%	23.7%	.06
DAS domain (carer-rated)			
Total	28.10 (11.64)	27.56 (11.49)	ns
Executive	3.90 (2.81)	5.67 (4.62)	ns
Emotional	10.30 (6.04)	9.48 (3.73)	ns
Initiation	14.30 (4.67)	12.94 (5.58)	ns
BBI post	11.83 (11.01)	9.93 (11.83)	ns
Normal	41.7%	52.5%	ns
Mild	41.7%	35.6%	ns
Severe	16.7%	11.9%	ns
ECAS baseline T1 (Version A; n)	11	57	
ECAS Total	103.20 (14.20)	104.32 (16.40)	ns
ALS-Specific	73.27 (21.85)	78.00 (13.34)	ns
Language	23.64 (6.47)	26.32 (5.16)	ns
Fluency	15.80 (4.85)	16.79 (4.63)	ns
Executive	33.55 (11.84)	36.36 (7.25)	ns
ALS-non-Specific	23.64 (6.47)	26.32 (5.16)	ns
Memory	12.91 (5.36)	14.61 (5.02)	ns
Visuospatial	11.09 (0.94)	11.71 (0.75)	.01*

* Significant after correction for multiple comparisons. All data reported in M(SD) unless otherwise stated.

Appendix 24 ECAS-HD (Chapter 10)

Rates of impairment on ECAS A – T1 and extended neuropsychological test domains.

ECAS A	Control		Premanifest		Manifest	
Domains	ECAS	FB	ECAS	FB	ECAS	FB
n	36		38		14	
ECAS Total	0.0%	0.0%	15.8%	5.3%	78.6%	64.3%
ALS-Specific	5.6%	0.0%	13.2%	0.0%	78.6%	50.0%
Language	0.0%	0.0%	14.7%	5.9%	53.8%	53.8%
Fluency	3.3%	0.0%	17.9%	0.0%	63.6%	27.3%
Executive	0.0%	0.0%	7.9%	2.6%	35.7%	35.7%
Non-Specific	2.9%	5.7%	26.3%	10.5%	58.3%	75.0%
Memory	2.9%	0.0%	18.4%	2.6%	58.3%	50.0%
Visuospatial	0.0%	8.6%	5.3%	21.1%	27.3%	90.9%

Table 6b: Proportion of abnormal scores on extended battery executive domains by group (ECAS A – T1).

Domains (T1)	Control	Premanifest	Manifest
Executive			
DSB	0%	13.2%	28.6%
RMET	2.8%	7.9%	61.5%
CWIT	0%	7.9%	66.7%

Impairment as <2SD published normative data.

Proportion of abnormal scores on extended battery executive domains by group (ECAS B – T2).

Domains (T2)	Control	Premanifest	Manifest
Executive			
DSB	0%	0%	0%
RMET	0%	0%	60%
CWIT Inhibition	0%	13%	50%

Impairment as <2SD published normative data.