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Neuropsychological and Neuropsychiatric
Endophenotypes in Amyotrophic Lateral Sclerosis

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degree of Doctor of Philosophy (PhD)

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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, unless stated otherwise, it is entirely my own work. Where any of the content presented is the result of input or data from related collaborative research this is acknowledged in the text.

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Finally, full and informed consent was obtained from all study participants.

Signed: Emmet Costello

Emmet Costello

This thesis is dedicated to all those living with ALS and their families.

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List of Abbreviations

ALS – Amyotrophic Lateral Sclerosis

ALSFRS-R – ALS Functional Rating Scale - Revised

AQ – Autism Spectrum Quotient

ASRS – Adult ADHD Self-Report Scale

AUDIT – Alcohol Use Disorders Identification Test

BBI – Beaumont Behavioural Index

BIS – Barrett Impulsiveness Scale

BNT – Boston Naming Test

CAPE – Community Assessment of Psychic Experiences

CR – Cognitive Reserve

CWIT – Colour-Word Interference Test

DAS – Dimensional Apathy Scale

D-KEFS – Delis-Kaplin Executive Functioning System

ECAS – Edinburgh Cognitive and Behavioural ALS Screen

EEG - Electroencephalography

EMG - Electromyography

FALS – Familial ALS

FrsBe – Frontal Systems Behavioural Scale

FTD – Frontotemporal Dementia

GAD – Generalised Anxiety Disorder

GHQ – General Health Questionnaire

GWAS – Genome-wide association study

HADS – Hospital Anxiety and Depression Scale

ICC – Intra Class Correlation

IGT – Iowa Gambling Task

LMN – Lower Motor Neurons

MND – Motor Neuron Disease

OCI-R – Obsessive-Compulsive Inventory Revised

PHQ – Patients Health Questionnaire

PLE – Psychotic-like experience

PSQ – Psychosis Screening Questionnaire

RAVLT – Rey Auditory Verbal Learning Test

RCFT – Rey Complex Figure Test

RCI – Reliable Change Index

RMET – Reading the Mind in the Eyes Test

SALS – Sporadic ALS

SART – Sustained Attention to Response Task

TIPI – Ten Item Personality Inventory

TOPF – Test of Premorbid Functioning

TOST – Two One-Sided t-test

UMN – Upper Motor Neurons

VFI – Verbal Fluency Index

WAIS – Wechsler Adult Intelligence Scale

WASI – Wechsler Abbreviated Scale of Intelligence

WMS – Wechsler Memory Scale

Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease, characterized by progressive muscle weakness and death, usually within 3-5 years from symptom onset. ALS is now recognized to be a multi-system disorder, affecting cognition and/or behaviour in up to 50% of patients.

Cognitive impairment is typically screened in ALS using the Edinburgh Cognitive and Behavioural ALS screen (ECAS). In study 1 of this thesis, the equivalence of alternative ECAS versions (i.e., ECAS A-B-C) and their practice effects were assessed in healthy controls (n = 236). Alternative ECAS versions were found to be highly comparable but not statistically equivalent. Serial administration of alternative versions produced small but significant practice effects.

Cognitive reserve (CR) i.e., the brains' ability to compensate for neuropathology, may moderate cognitive impairment in ALS. CR is protective against cognitive symptoms in Alzheimer's, Huntington's, and Parkinson's. However, CR has yet to be examined in ALS. In study 2 of this thesis, the association between CR (measured using education, occupation and physical activity) and cognitive decline in ALS patients (n = 189) was assessed. CR was associated with better verbal fluency and language performance. CR was also associated with better baseline performance in memory, but a sharper decline over time, suggesting CR is more protective for ALS associated impairments.

The most prevalent genetic cause of ALS is a mutation of the C9orf72 hexanucleotide repeat expansion, however this mutation only accounts for ~40% of Familial ALS (FALS) and ~10% of Sporadic ALS (SALS), meaning the genetic cause of most ALS cases remains unknown. Genetic studies may fail to identify risk genes because they are overly reliant on clinical description to define a phenotype. Endophenotypes (i.e., quantitative traits which lie between an illness and its underlying genetic cause) may offer a more promising approach to gene discovery as they can help identify individuals at risk prior to disease onset and provide greater statistical power in localizing and identifying disease related genes.

In study 3 of this thesis, first- and second-degree relatives of ALS patients (n = 224) and healthy controls (n = 134) were recruited to examine endophenotypes in ALS. Participants were administered a neuropsychological assessment, a blood sample was taken for genetic testing, and participants were given a neuropsychiatric questionnaire to complete online.

ALS relatives performed significantly worse than controls on executive functioning, language, memory and IQ domains, with large effect sizes for verbal fluency and confrontation naming tasks. Deficits were greater in relatives of FALS patients, likely due to a greater genetic burden in these individuals. ALS relatives were also characterized by initiation apathy, greater autism traits, low openness to experience and low conscientiousness traits. IQ deficits were a central component of the cognitive endophenotype in ALS relatives, but did not fully account for verbal fluency and

confrontation naming deficits. Neuropsychological and neuropsychiatric outcomes clustered into two distinct sub-groups, potentially indicative of diverging risk profiles in ALS kindred.

Overall, these findings suggest 3 promising candidate endophenotypes for ALS: verbal fluency, confrontation naming and openness to experience traits. Future research is needed to validate these endophenotypes on a molecular and genetic level and determine their usefulness towards gene discovery.

Peer-reviewed publications from this thesis

1. Costello E, Lonergan K, Madden C, O’Sullivan M, Mays I, Heverin M, Pinto-Grau M, Hardiman O, Pender N. Equivalency and practice effects of alternative versions of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2020 Jan 2;21(1-2):86-91.
2. Costello E, Rooney J, Pinto-Grau M, Burke T, Elamin M, Bede P, McMackin R, Dukic S, Vajda A, Heverin M, Hardiman O. Cognitive reserve in amyotrophic lateral sclerosis (ALS): a population-based longitudinal study. *Journal of Neurology, Neurosurgery & Psychiatry*. 2021 May 1;92(5):460-5.

Presentations from this thesis

1. Costello, E., Ryan, M., McHutchinson, C., ... & Hardiman, O. (2018). Endophenotypes in ALS: Cognitive and psychiatric assessment of unaffected relatives of ALS patients. Presented at the Annual European Network for a Cure for ALS (ENCALS) in Tours, France.
2. Costello, E., Ryan, M., Rooney, J., ... & Pender, N. (2019). Cognitive reserve as a mediator of cognitive decline in Amyotrophic Lateral Sclerosis. Presented online at the Annual Motor Neuron Disease Association Symposium in Perth, Australia. Also presented online at the Psychology Society of Ireland Annual Conference. Awarded

highly commended in the Division of Neuropsychology Early Career Award.

1. Chapter 1: An Introduction to Amyotrophic Lateral Sclerosis and Endophenotypes

1.1. Background of ALS

1.1.1. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition characterized by progressive degeneration of the upper and lower motor neurons, resulting in a relentless, progressive paralysis and death (1). ALS is the most common form of Motor Neuron Disease (MND), a group of disorders which also include Primary Lateral Sclerosis (PLS), Progressive Bulbar Palsy (PBP), Pseudobulbar Palsy, Progressive Muscular Atrophy (PMA) and Spinal Muscular Atrophy (SMA). There is currently no cure for ALS, although symptoms can be managed and survival prolonged through multidisciplinary care (2) (3). Individuals with ALS typically die as a result of respiratory failure within 3 years of disease onset (1).

1.1.2. ALS is not solely a disease of the motor system. Extra-motor pathology in frontal, prefrontal and temporal regions is commonly found upon histopathological examination (4). Extra-motor involvement is associated with impairment in executive functioning and behavioural dysfunction, with 14% of ALS patients also meeting criteria for co-morbid Frontotemporal Dementia (FTD) (5). There is now growing recognition that ALS and FTD represent two sides of a disease spectrum, known as ALS fronto-temporal spectrum disorder (FTSD) (6).

1.1.3. Clinical features

1.1.3.1. The signature hallmark of ALS is progressive motor weakness, without sensory dysfunction (4). In ~60% of patients, their first symptom is weakness in a limb (i.e., limb onset), ~30% first notice difficulty with speech (i.e., bulbar onset), ~5% initially have cognitive and/or behavioural change (i.e., cognitive/behavioural onset), and ~5% will first have respiratory weakness (respiratory onset) (4) (see figure 1.1.).

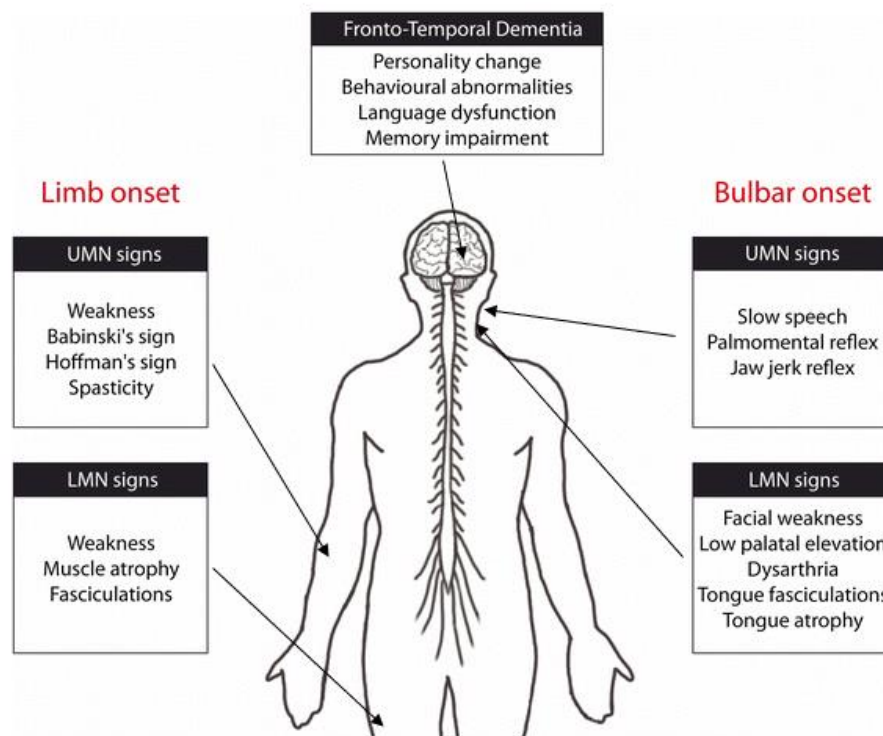


Figure 1.1. Clinical features of limb onset and bulbar onset ALS. Adapted from Picher-Martel et al. (7)

1.1.4. Diagnosis

1.1.4.1. ALS is diagnosed by establishing a history of progressive motor dysfunction over time. As a result, ALS is associated with a long diagnostic delay, typically 10-16 months from symptom onset (8). Over this time a

neurologist must identify progression of both upper and lower motor neuron signs.

1.1.4.2. Upper motor neuron (UMN) signs include dysarthria (speech difficulty), dysphagia (swallowing difficulty), sialorrhea (excessive salivation), pseudobulbar affect (uncontrollable laughing or crying) and laryngospasm (spasm of the vocal cord) (9).

1.1.4.3. Lower motor neuron (LMN) signs include muscle atrophy (weakness), fasciculations (twitches) and cramps. LMN signs are often investigated using electromyography (EMG) (9). During an EMG, an electrode needle is inserted into the muscle, testing its electrical activity when contracted and at rest. EMG is ~60% sensitive and therefore cannot be used in isolation (4).

1.1.4.4. As ALS is a diagnosis of exclusion, the presence of sensory findings, sphincter involvement or a significant movement disorder should lead to additional investigations. Clinicians may undertake nerve conduction studies, Magnetic Resonance Imaging (MRI), blood and urine tests, lumbar puncture and muscle biopsy in order to rule out other conditions, such as: multifocal motor neuropathy, spinal muscular atrophy, polyradiculopathy, myopathies and neuromuscular transmission disorders (9).

1.1.4.5. Depending on these investigations, and the resulting evidence of ALS, a clinician may diagnose ALS according to consensus diagnostic criteria. Over time, El Escorial (10), El Escorial-revised (11), Airlie House (12), Awaji (13) and Gold Coast (14) criteria have been developed and

refined to standardize the diagnosis of ALS. The El Escorial-Revised criteria are the most commonly applied, classifying ALS diagnosis according to degree of certainty. The diagnostic criteria are as follows:

Definite ALS: progressive UMN and LMN signs in 3 of 4 regions (bulbar, cervical, thoracic and lumbosacral spinal cord).

Probable ALS: progressive UMN and LMN signs in 2 of 4 regions.

Clinically probable – lab supported ALS: UMN and LMN signs in 1 region, or UMN signs alone in 1 region or LMN signs in at least two limbs. These must be in conjunction with neuroimaging and/or clinical laboratory tests that rule out alternative diagnoses.

Clinically possible ALS: UMN and LMN signs in 1 region, or UMN signs in at least 2 regions or LMN signs rostral to UMN signs.

1.1.4.5. Whilst not specified in the diagnostic criteria, cognitive and behavioural impairment are now recognized as common features of ALS and can even precede motor involvement. The revised Strong criteria (6) provide a framework for diagnosing ALS with varying degrees of cognitive and behavioural impairment (see table 1.1.).

Table 1.1. Revised Strong criteria, modified table taken from Strong et al. (6).

ALS subtype	Characteristics
ALSbi	1. Apathy with or without other behaviour change <u>OR</u> 2. meeting at least two non-overlapping diagnostic features from Rascovsky criteria (15).
ALSci	Evidence of either executive or language dysfunction or a combination of the two. Executive impairment is defined as: Impaired verbal fluency <u>OR</u> impairment on two independent measures of executive functions. Language impairment is defined as: Impairment on two independent tests of language.
ALSchi	Patients who meet the criteria for both ALSci and ALSbi.
ALS-FTD	1. Evidence of progressive deterioration of behaviour and/or cognition by observation or history <u>AND</u> 2. At least 3 behavioural/cognitive symptoms outlined by Rascovsky (15) <u>OR</u> 3. At least 2 of those behavioural/cognitive symptoms, with loss of insight and/or psychotic symptoms <u>OR</u> 4. Language impairment meeting criteria for semantic dementia/semantic variant PPA or non-fluent variant PPA.
ALS-dementia	ALS with dementia, not typical of FTD.
ALSbi – ALS with behavioural impairment; ALSci – ALS with cognitive impairment; ALSchi – ALS with cognitive and behavioural impairment	

1.1.4.6. ALS diagnosis can also be categorized based on whether a patient has a family history of ALS or FTD. Globally, ~85-95% of individuals with ALS have no family history of ALS or FTD, and are thereby referred to as sporadic ALS cases (SALS) (16). Globally, ~5-15% of individuals with ALS

report that they have a family member who had ALS or FTD, and are thus referred to as familial ALS cases (FALS) (16). In Ireland, the recognition of familial ALS has grown steadily from 1994 to 2016, and is now estimated between 20-30% depending on the criteria used (17). This increased rate of FALS is likely because Ireland has the world's longest running ALS register, allowing for greater case ascertainment, rather than due to a truly increased prevalence of FALS.

1.1.5. Disease progression

1.1.5.1. Disease progression in ALS is considerably heterogeneous. After initial symptoms have emerged in one of 4 primary regions (bulbar, thoracic, cervical or lumbosacral), the disease spreads, eventually encompassing all 4 regions. The Kings (18) and Milano-Torino (MiToS) (19) staging systems were developed to operationally define disease progression into distinct clinical stages. These stages (summarized in figure 1.2. (20)) assist clinicians in monitoring disease severity and help guide treatment, e.g., when to introduce non-invasive ventilation or feeding tube.

King's clinical staging system



MiToS functional staging system

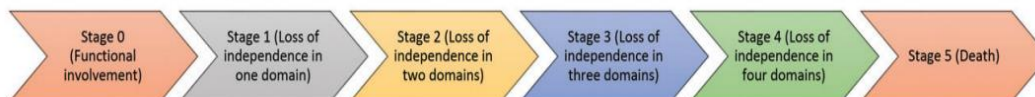


Figure 1.2. Flowchart of Kings and MiToS staging systems, taken from Fang et al. (20).

1.1.5.2. Functional decline in ALS is typically quantified using the ALS Functional Rating Scale - Revised (ALSFRS-R). The ALSFRS-R is a 12-item questionnaire that assesses bulbar, spinal and respiratory symptoms. There are currently no established biomarkers for disease progression in ALS. As a result, the ALSFRS-R total score is commonly used as a proxy for disease progression in clinical trials and research. However, a large scale longitudinal study of Irish ALS patients found that ALSFRS-R sub-scores are more valid prognostic indicators than ALSFRS-R total score (21).

1.1.6. Treatment

1.1.6.1. Riluzole is the only approved medication for ALS in Europe. Riluzole has been shown to lengthen survival by 2-3 months, although the exact mechanism by which it does this remains unclear (22). Riluzole blocks TTX sodium channels implicated in damaged neurons and reduces glutamate-induced excitotoxicity. A new drug, Edaravone, has recently been approved to treat ALS in the US, following a successful phase 3 randomized control trial (23). Edaravone was found to mitigate functional decline as measured by ALSFRS-R in a small subgroup of patients. However, the clinical meaningfulness of this reduced decline, and the limited group in which it was effective in, raises concerns as to its usefulness.

1.1.6.2. Multidisciplinary team (MDT) care is essential in ensuring ALS patients get the best possible health and quality of life outcomes. This involves the close collaboration of neurologists, specialist nurses, neuropsychologists, speech and language therapists, physiotherapists and occupational therapists working in tandem with patients and their families

(see table 1.2. for a summary of each professions role in patient care). In Ireland, there is a weekly multidisciplinary clinic where patients access clinical care. Patients can attend all team members on the same day in the one clinic, resulting in an enriched clinic experience that improves outcomes. MDT care has been proven to extend survival in Ireland and the UK (24) (25) and is now considered best practice.

Table 1.2. The role of each member of the Multi-Disciplinary Team.

Profession	Role in the MDT
Neurologist	Diagnosis, treatment and symptom management, initiation of nutritional and respiratory interventions.
MND Specialist Nurse	Liaison with MDT and coordination of care, home visits,
Neuropsychologist	Assessment and management of cognitive, behavioural and emotional difficulties.
Speech and Language Therapist	Assessment and management of dysphagia and aspiration, facilitating communication needs.
Occupational Therapist	Management of the patient environment, providing adaptive devices and information on patient safety.
Dietician	Monitors nutritional status, management of dysphagia, assesses need for nutritional intervention.
Physiotherapist	Examines muscle strength and mobility, provides orthoses and
Respiratory Physician	Evaluates respiratory functioning, advises need for non-

1.1.6.3. Muscle spasms and cramps can be managed using Quinine sulphate (26). Baclofen and tizanidine can address muscle spasticity. Sialorrhea (excessive saliva) can be treated using amitriptyline, atropine, hyoscyamine, glycopyrronium or botulinum toxin injection. Pain is managed using anti-inflammatories, anti-convulsants and opiates if necessary. Emotional lability can be reduced with amitriptyline or fluvoxamine.

1.1.6.4. Recent advancements in antisense oligonucleotide (ASO) therapy offers new hope for an effective treatment for ALS (27). Antisense therapy targets messenger RNA, altering the expression of a gene. Antisense therapy has shown efficacy in Spinal Muscular Atrophy (28); and clinical trials for genetically mediated ALS have recently begun (27). The promise of these therapies highlights the clinical importance of gene discovery and the accurate characterization of symptomatic and asymptomatic gene carriers.

1.1.7. Cognitive and behavioural impairment in ALS

1.1.7.1. Once believed to be a purely motor disease, it is now well established that cognitive and/or behavioural impairment is often present in ALS. Approximately 15% of ALS patients will meet criteria for co-morbid Fronto-Temporal Dementia (FTD) (5), a neurodegenerative disease characterized by changes in personal and social behaviour, emotional blunting, apathy and language deficits (29). ALS and FTD share genetic and neuropathological underpinnings, and are now seen as two sides of a disease spectrum (30). A mutation of the C9orf72 repeat expansion is a risk factor for both conditions; and the pathological substrate TDP-43 is found in 97% of ALS patients and 45% of FTD patients (31) (see figure 1.3.).

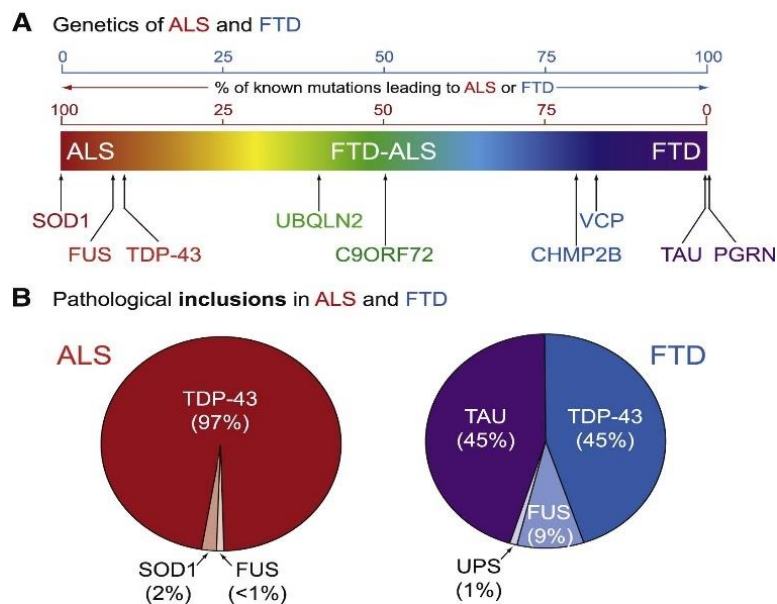


Figure 1.3. Overlapping (A) genetic and (B) neuropathological features of ALS and FTD, taken from Ling et al., (30).

1.1.7.2. In addition to those with co-morbid FTD, ~30% of ALS patients will experience cognitive and/or behavioural impairment (see figure 1.4.) (5). A comprehensive systematic review and meta-analysis of cognition in ALS, suggests that there is strong evidence for deficits in language, verbal fluency, executive functioning and memory in ALS (32). Medium effect sizes were found for verbal fluency, language and social cognition tasks, while small effect sizes were observed on delayed verbal memory and executive functioning tasks. However, due to the limited number of studies on various domains, confidence intervals were wide, making it difficult to determine an accurate estimate of true effect sizes. Furthermore, this meta-analysis was carried out solely on cross-sectional studies.

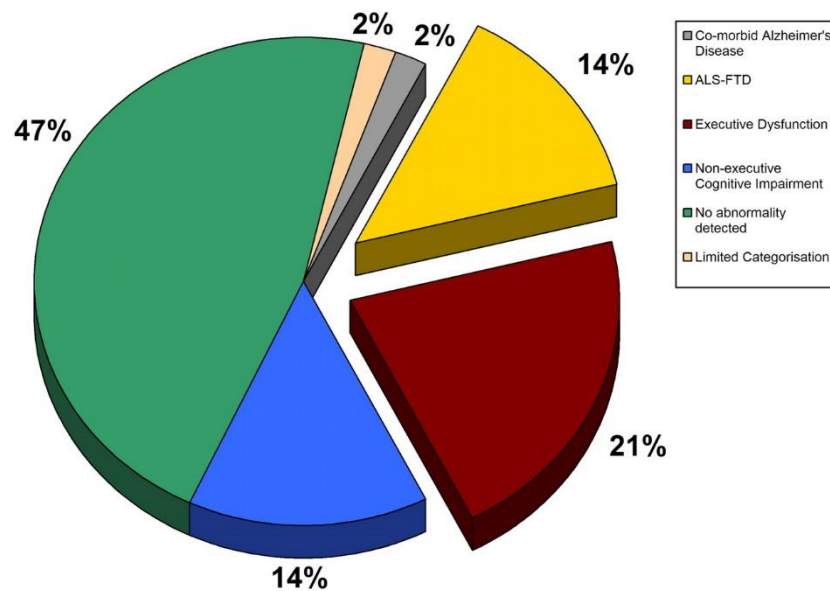


Figure 1.4. Prevalence of each type of cognitive impairment in ALS. Taken from Phukan et al. (5)

1.1.7.3. Cognitive impairment is commonly assessed using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (33). The ECAS is designed to account for motor impairment in ALS and is broken down into 5 cognitive domains: language, verbal fluency, executive, memory and visuospatial domains. Language, verbal fluency and executive domains are summed to give an ALS specific score. This is called ALS specific because these tests are sensitive to the impairments seen in ALS. Memory and visuospatial functioning are summed to give an ALS non-specific score. This score is deemed ALS non-specific because these functions are relatively spared in ALS, although memory deficits have been observed in ALS (32).

1.1.7.4. To facilitate longitudinal assessment of cognition in ALS, alternative versions of the ECAS (ECAS B and C) were developed (34). Initial analysis of the 3 ECAS versions suggests that they do not produce significant

practice effects (35). However, ECAS B and C have yet to be validated in an Irish population. Furthermore, Irish-specific abnormality thresholds are needed for ECAS versions B and C to optimally screen for cognitive impairment in Irish ALS patients.

1.1.7.5. Behavioural impairment is commonly assessed in ALS using the Beaumont Behavioural Index (BBI) (36). The BBI is a questionnaire given to a family member of a patient where they are asked to rate if the patient has changed in respect to certain behaviours. The questionnaire specifically asks about behaviour changes commonly seen in ALSbi. These include apathy, impulsiveness, disinhibition, psychotic experiences and dysexecutive behaviour.

1.1.7.6. In cases where ECAS performance is abnormal (i.e., below norm-generated cut-offs) or a caregiver is reporting severe behavioural change on the BBI, a patient is referred for a full neuropsychological assessment. The neuropsychological assessment consists of an in-depth battery of tests and a clinical interview where the neuropsychologist examines specific cognitive and behavioural deficits and provides feedback to the patient and their family. A clear understanding of an individual's cognitive and behavioural status provides the patient with a better understanding of their illness and allows clinicians to tailor their treatment to ensure the patient is able to make informed healthcare decisions.

1.1.7.7. Two recent studies of Irish, Scottish, English and Italian cohorts suggest that cognitive impairment is more frequent in later disease stages (37) (38). A cross-sectional study of Irish, Scottish and English individuals

with ALS found that 80% of patients had mild or severe cognitive impairment at stage 4 of the disease (according to King's staging) (37), while in the Italian cohort, this figure was 64% (38). However, a major limitation of both studies is that they are cross-sectional rather than longitudinal, and therefore do not capture cognitive decline within participants.

1.1.7.8. Most longitudinal studies of cognition in ALS suggest that impairment tends to emerge early in the disease (39) (40) (41). A population-based, incident study of Irish ALS patients found that those without impairment at baseline, tended to remain cognitively normal throughout their illness (39). In contrast, patients who were impaired at baseline declined thereafter. The subgroup of ALS patients with cognitive decline had the highest rate of attrition due to death or disability, illustrating why it is so difficult to capture cognitive decline longitudinally. Patients with cognitive impairment also had a faster functional decline and shorter survival, indicating that cognitive impairment is a risk factor for shorter survival in ALS (39).

1.1.7.9. A recent longitudinal study in Italy found that 32% of the sample showed a decline in cognitive status (42). Contrary to the Irish cohort study, even those who were normal at baseline showed evidence of decline. This study was highly powered; however, it was limited to just a 6-month follow up and only 2 assessments. Due to the rapidly progressive nature of the ALS, attrition is high due to death or advanced disease stage. This likely biases previous estimates of the true rate cognitive and behavioural decline over time.

1.1.7.10. The contrasting findings of cross-sectional and longitudinal studies leaves uncertainty as to the true extent to which cognition declines in ALS patients, particularly for those who are unimpaired at baseline. Cross-sectional studies comparing disease stages suggest cognitive impairment is more evident in more advanced disease stages. This may, in part, be due to the nature of the staging systems applied. Bulbar onset patients make up a higher proportion of stage 3 and stage 4 patients and may be more vulnerable to cognitive impairment (43). This would mean that cognitive decline is simply more common in bulbar patients, rather than that cognitive change occurs in tandem with disease stage. When patients have been studied longitudinally, decline is limited to a sub-group who were impaired at baseline. Cross-sectional studies are limited by their lack of longitudinal follow up to track cognitive change in individuals, while longitudinal studies are limited by their high attrition rate.

1.1.7.11. A novel method that may overcome the limitation of high attrition is joint modelling. Joint longitudinal and time-to-event modelling (referred to simply as ‘joint modelling’) has recently gained popularity as a means of limiting the effect of non-random drop-out (e.g., attrition due quicker disease progression) on longitudinal outcomes (44). A joint model approach may help to clarify the question surrounding longitudinal change in cognition in ALS.

1.1.7.12. A potential moderator of cognitive change, that may account for some of the varied presentations in ALS, is cognitive reserve (CR). Theories of CR stipulate that enriching experiences across the lifespan, such as

education, mentally challenging occupations and rich social interactions increase an individual's neural resources (e.g., greater grey matter volume or white matter tract integrity) (45) (see figure 1.5.). An evolving model of the disease is that as spread occurs across networks, individuals may recruit reserve neural resources to deal with cognitive demands, a process known as compensation.

1.1.7.13. There is some evidence that compensatory processes occur in ALS. Neuroimaging findings (specifically PET) show that ALS patients with cognitive impairment showed reduced white matter volume in fronto-temporal association fibres (46). Patients who are not cognitively impaired also showed evidence of white and gray matter changes, suggesting the presence of compensatory mechanisms (47). This compensatory mechanism may account for the increased connectivity (i.e., increased neural communication) observed in ALS patients (47). CR is protective against cognitive impairment in numerous neurodegenerative conditions, such as Parkinson's disease, Huntington' disease and dementia (48–52), however, it has yet to be studied in ALS.

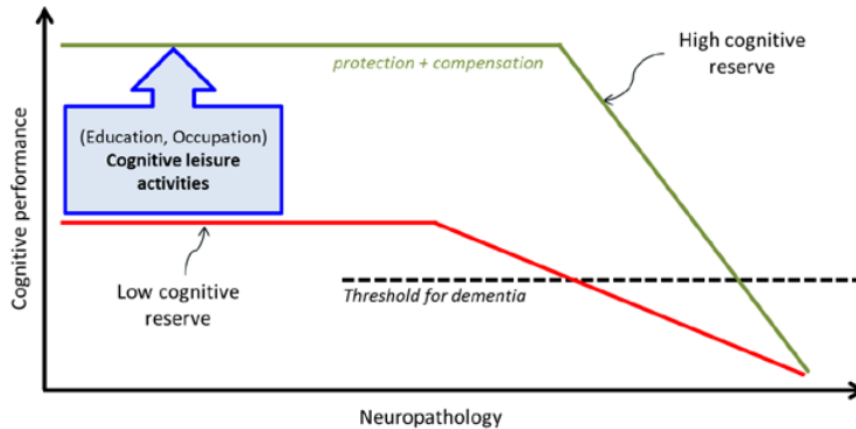


Figure 1.5. Hypothesized model of how cognitive reserve moderates the association between neuropathology and cognitive performance. Taken from Mistridis et al. (53)

1.1.7.14. A significant proportion of ALS patients will experience behavioural change but do not meet the criteria for co-morbid FTD. Apathy is the most commonly reported change, present in ~28 - 43% of ALS cases (54). Specifically, patients experience increased initiation apathy, i.e., they show less self-generated thoughts and behaviours, even after controlling for motor impairment. Other less-common behavioural changes include irritability, disinhibition, emotional blunting and a lack of empathy (55). A major challenge in identifying behavioural change in ALS patients is that they are typically quantified using caregiver reports and questionnaires. As a result, these measures are less objective than performance-based metrics.

1.1.7.15. An Irish ALS cohort study found that behavioural features of ALS appear to cluster, forming 5 distinct patient sub-phenotypes (56), which reflect dysfunction in distinct brain networks. Group 1 was characterized by disinhibited behaviour, reflective of disruption to the medial prefrontal and

orbitofrontal cortices and the anterior insula. Group 2 was characterized by impaired impulse control, reflective of dysfunction to orbitofrontal and anterior cingulate pathways. Group 3 was marked by dysexecutive behaviour, reflective of right prefrontal changes. Group 4 was distinguished by notable cognitive rigidity, reflecting dorsolateral prefrontal cortex and temporo-limbic network dysfunction. Group 5 was characterized by severe behavioural change across all behavioural domains to the extent that they would meet criteria for co-morbid FTD.

1.1.7.16. Behavioural change in ALS patients has a significant impact on the degree of burden to their caregivers (57), even more so than a patients physical disability. Caregivers of patients with behavioural change are 1.4 times more likely to experience high caregiver burden, with impulsivity and disinhibition the biggest causes of burden (58). Co-morbid FTD and co-morbid executive functioning are also significant negative prognostic indicators, associated with survival hazards ratios of 2.67 and 3.44 respectively (59). This may be due to reduced compliance with treatments such as non-invasive ventilation, or indicative of a faster disease process in these patients.

1.2. Epidemiology of ALS

1.2.1. Globally, the incidence of ALS is estimated to be between 0.6 and 3.8 persons per 100,000 (60). Incidence refers to the rate at which an event or disease occurs and is quantified as the number of new cases of a specific disease occurring during a particular period in a particular population. Incidence is an important metric to estimate disease burden and to compare

disease burden in varying populations. Prevalence is the proportion of a population who are affected by a particular disease. It is calculated by comparing the number of people who have an illness to the total population. Globally, the prevalence of ALS is estimated to be ~5.2 persons per 100,000 (61).

1.2.2. Few studies on incidence have been carried out outside of Europe. In Beijing, the estimate is 0.8 per 100,000 (62), and in South Korea it's 1.2 per 100,000 (63). This may be due to a lower prevalence of ALS risk genes in Asian populations (60). There is also growing evidence that ALS is less common in countries with greater admixture, i.e., more genetic variation, such as Cuba (64).

1.2.3. The reported incidence of ALS appears to be rising (60). In Scotland, the incidence of ALS increased 36% over a 25 year time period (65). However, increasing incidence is likely due to greater ALS ascertainment and improved survival in competitive diseases. There is no definitive evidence that the true rate of ALS (adjusted for age) is increasing over time (1).

1.2.4. In Ireland, the incidence of ALS is 2 persons per 100,000 and the prevalence is 6.4 persons per 100,000 (66). This equates to ~140 new diagnoses of ALS a year, and ~300-500 people living with ALS in Ireland at any one time.

1.3. Environmental risk factors

1.3.1. Identifying environmental risk factors for ALS poses several challenges. Unlike the genome, the exposome (i.e., everything a person is exposed to) is infinite in both time and space. Environmental studies are expensive, requiring in-depth measurement and large cohort sizes. The following section outlines the most studied environmental risk factors for ALS.

1.3.2. Exercise

1.3.2.1. ALS patients are often noted for having high premorbid fitness (67). This perception has been driven in part by high profile athletes who've developed ALS, such as the legendary baseball player Lou Gehrig, and more recently with rugby players Doddie Weir and Rob Burrow. A study of soccer players from the top Italian leagues observed an increased incidence of ALS, with standardized morbidity risk estimates between 6.5 and 11.6 (68). These findings were not replicated in a similar study of cyclists, indicating a specific effect of soccer (69). However, the higher risk reported in soccer players may be due to how the risk was calculated. If lifetime risk is applied, rather than incidence rate, an increased risk in soccer players is no longer observed.

1.3.2.2. Epidemiological studies have shown higher levels of varsity athleticism in ALS patients in the US (69). A population-based study in the Netherlands found that ALS patients reported slightly greater levels of physical activity but not significantly higher engagement in extreme exercise

(e.g., marathon running) (70). This suggested that exercise may be a marker of risk, rather than a causative factor.

1.3.2.3. Two recent studies suggest that the relationship between exercise and ALS is more nuanced. A Mendelian randomization experiment found that exercise was associated with motor neuron injury, but only for those with known risk genes (71). Transcriptomic analysis found that ALS risk genes are activated in response to physical exercise. This was particularly the case for people who carry the C9orf72 repeat expansion, where age of onset was inversely proportional to historical physical activity levels. A large scale, longitudinal case-control study in the Netherlands found that patients with C9orf72 repeat expansion mutation did not have significantly greater premorbid physical activity levels, but did have significantly lower BMI (72). Together, these findings indicate that exercise may be a causal risk factor for a subset of individuals with a specific genotype.

1.3.3. Smoking

1.3.3.1. The effect of smoking on ALS risk has seen mixed results. Some studies have shown evidence of an increased risk of ALS in smokers (73), while others have found no such association (74). A meta-analysis of 20 studies concluded that there is no strong evidence of an association between smoking and ALS risk in men, however, women smokers had a higher risk of ALS (relative risk = 1.31 - 2.10) (75).

1.3.4. Pesticides and chemicals

1.3.4.1. Organic solvents, agricultural chemicals and pesticides have been explored as potential risk factors for ALS. A systematic review of 38 studies

indicated a small increase in ALS risk as a result of pesticide exposure (76). However, very few studies were of high methodological quality, and more well-designed studies are needed.

1.3.5. Occupation

1.3.5.1. Occupations such as veterinarians, athletes, hairdressers, power production plant operators and armed forces personnel have been investigated as potential risk factors for ALS (77). However, few studies have been of good methodological quality. Gulf war veterans suffer from increased rates of ALS, suggestive of a war-related environmental trigger (78). However, a review by the U.S. Institute of Medicine rebuked this association (79).

1.3.6. Geographical location

1.3.6.1. Clustering of ALS has been observed in Guam, a small island in the western Pacific, and in the Kii peninsula, in Japan. The phenotype observed in these locations was atypical, with a large degree of parkinsonian and dementia features. Clusters of ALS have also been reported in Spain, Finland, UK, USA and Italy. However, no common factors between these geographical locations have been identified that might account for these higher rates. The high incidence of ALS in these regions may be because of founder effects (80). This refers to the loss of genetic variation that occurs when a new population is established by a small number of individuals.

1.3.6.2. An analysis of the Irish ALS population failed to identify any hotspots (i.e., areas of high incidence), however it did identify two cold spots (i.e., areas of low incidence) in Kilkenny and Clare (81). Lower ALS

incidence in these regions may be due to historical events such as Viking settlements in Waterford or the forced Cromwellian transplantations in Clare which led to greater admixture (i.e., greater genetic variation) in these regions. The particularly low incidence in the Burren in Clare may be attributable to the notable karst landscape in the region.

1.3.7. Cyanotoxins

1.3.7.1. B-N-methylamino-L-alanine (BMAA), a toxin in the cycad tree, was hypothesized to be the key contributing factor to the high incidence of atypical ALS in Guam (82). BMAA was also proposed as the underlying cause of high ALS rates in Gulf war veterans as it is abundant in cyanobacterial crusts in the gulf desert (83). While BMAA has been studied extensively, research has yet to find any definitive relationship between BMAA and ALS (84).

1.4. Neuropathology

1.4.1. TDP-43 inclusions

1.4.1.1. 43 kDa Tar-DNA binding protein (TDP-43) misfolding and accumulation is found in the brain and spinal cord of 97% of ALS patients, the exception being those with the superoxide dismutase 1 (SOD1) mutation (31). TDP-43 is predominately a nuclear protein (found in the nucleus of the cell) and is involved in transcriptional regulation (the process by which the cell converts DNA to RNA). In ALS, TDP-43 is phosphorylated, i.e., a phosphoryl group is added to a molecule (phosphorylation is critical for many cell functions, particularly proteins, where phosphorylation activates

almost half of enzymes), and C-terminally truncated, i.e., the end of amino acid is cut short in cytoplasmic aggregates.

1.4.1.2. This phosphorylation process may lead to either a pathological loss or gain of function in TDP-43 proteins. Insoluble aggregates inhibit TDP-43's function in transcriptional regulation, dysregulate proteostasis (the complex biological systems that regulate protein creation and folding) and sequester other aggregation prone proteins, resulting in further cytotoxicity and cell death.

1.4.2. TDP-43 and cognitive impairment

1.4.2.1. Recent findings suggest that cognitive impairment is a strong predictor of TDP-43 pathology post-mortem (85). Furthermore, the type of impairment (language, executive, fluency) was predictive of the regions where TDP-43 was present. In some cases, patients had extensive TDP-43 pathology, yet cognition remained intact (so called mismatch cases). This may suggest that these patients are able to withstand TDP-43 pathology through compensatory mechanisms or cognitive reserve. All patients with the mutated C9orf72 repeat expansion (see section 1.7.1.) were found to have TDP-43 pathology in non-motor brain regions.

1.5. Neuroimaging

1.5.1. Standard computerized tomography (CT) or magnetic resonance imaging (MRI) scans are unable to detect structural changes in ALS. However, advanced techniques, such as functional MRI (fMRI), positron emission tomography (PET), diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (MRS), are able to detect focal gray and

white matter loss and reductions in white matter integrity (86). Degeneration can be seen in numerous brain regions, such as the motor cortex, frontal lobe, basal ganglia, corpus callosum, corticospinal tract, brainstem, thalamus and cervical spinal cord.

1.5.2. Extra-motor involvement is more severe in ALS patients with cognitive and/or impairment, particularly in the temporal lobes and white matter tracts (87). Diffusivity abnormalities in the corpus callosum and the fronto-temporal tracts are the best predictors of cognitive and/or behavioural impairment in patients.

1.5.3. ALS patients with the C9orf72 repeat expansion mutation display a distinct neuroimaging profile, characterized by fronto-temporal, hippocampal, thalamic and insular changes. These cortical and sub-cortical abnormalities are also observed in asymptomatic C9orf72 gene carriers under 40 years of age (88). This suggest that the underlying neuropathology of ALS may begin much earlier than previously thought, and raises the possibility that C9orf72-mediated ALS could be considered a neurodevelopment disorder (89).

1.6. Electrophysiology

1.6.1. Electroencephalography (EEG), while limited in terms of spatial resolution, offers superior temporal resolution than MRI/fMRI, allowing for in-depth characterization of brain networks. EEG studies have consistently shown increased fronto-parietal connectivity in ALS patients relative to controls, specifically in γ -frequency (gamma) bands (90) (47). Source localisation analysis, which improves spatial resolution in EEG, indicates

that ALS patients have decreased spectral power in occipital, temporal, orbitofrontal and sensorimotor regions (91). ALS patients also show increased central – posterior and central – frontal co-modulation, and decreased temporal – frontal and temporal – sensorimotor synchrony.

1.6.2. Event related potentials (ERP's) measure the brains' immediate reaction to a sensory, cognitive or motor event. As a result, they are able to capture millisecond by millisecond disruption to executive networks (92). ALS patients display a distinct ERP profile using a mismatch negativity (MMN) paradigm, a measure of involuntary attention switching networks (93). ALS patients display a significantly delayed MMN response, which correlates with cognitive performance on the Stroop task, a measure of inhibitory control and attention switching. Source localisation under this paradigm suggests that the deficits seen in inhibitory control are underpinned by excessive left fronto-parietal activity and decreased inferior frontal activity (94).

1.7. Genetics of ALS

1.7.1. C9orf72 repeat expansion

1.7.1.1. The most prevalent genetic cause of ALS and FTD in populations of European extraction is a mutation of the C9orf72 hexanucleotide repeat expansion (95) (see figure 1.6). Linkage and genome wide association (GWA) studies of ALS and FTD families first found that a locus on chromosome 9p21 was linked to ALS-FTD (96). Subsequently, an expanded hexanucleotide repeat (GGGGCC) was identified in the region C9orf72 as the cause of chromosome 9p21 linked ALS-FTD. Globally, C9orf72 is

estimated to be present in ~60% of familial ALS cases and 10% of sporadic ALS cases (97) (98). The exact number of hexanucleotide repeats considered to be pathological is still unclear. Studies have typically marked more than 23 repeats as indicative of pathology (97).

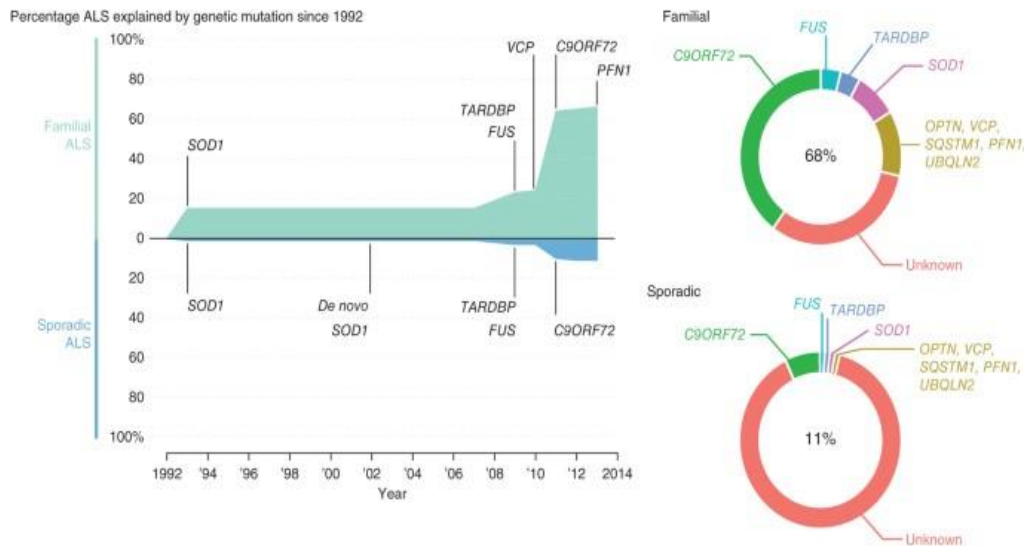


Figure 1.6. The proportion of ALS explained by each gene in populations of European ancestry. Taken from Renton et al. (80)

1.7.2.2. C9orf72 repeat expansions result in either a pathological loss or gain of gene function. This leads to a build-up of neuronal aggregates of nuclear RNA foci, dipeptide repeats (DPR) and TDP-43 inclusions (99). RNA foci are present across the central nervous system. They are found in neuronal nuclei, particularly in the hippocampus, cerebellum, spinal cord and motor and frontal cortex (100). DPR inclusions are found in the cerebellum, hippocampus and neocortex (101). TDP-43 inclusions are observed in the temporal, frontal and primary motor cortex, as well as the amygdala, basal ganglia, thalamus, midbrain and hippocampus (100).

1.7.1.3. In Ireland, the C9orf72 repeat expansion is observed in ~41% of familial cases and ~5% of apparently sporadic cases (102), displaying a distinct clinical phenotype. A positive C9orf72 status is associated with earlier disease onset, a higher likelihood of cognitive and behavioural impairment, a specific neuroimaging profile characterized by non-motor cortex changes, a family history of neurodegenerative diseases with autosomal inheritance and reduced survival. This profile has been replicated in numerous cohorts, including Italian (103), Dutch (104) and American (105) populations.

1.7.2. SOD1

1.7.2.1. Cu/Zn Superoxide dismutase (SOD1), was the first causative gene linked to ALS (106). The SOD1 gene codes to produce homodimeric metalloenzyme, an enzyme responsible for the conversion of O_2^- into O_2 and H_2O_2 , a process known as the Fenton reaction. SOD1 is typically inherited though dominant inheritance and accounts for ~12% of familial ALS and ~1% of sporadic ALS (80).

1.7.2.2. Individuals with a SOD1 mutation typically have a younger onset, longer disease duration, a lower limb site of onset and less frequent cognitive and behavioural impairment (107). SOD1 patients are one of the only patient groups who do not display evidence of TDP-43 aggregates, raising doubt about the validity and value of SOD1 ALS mouse models.

1.7.3. TARDBP

1.7.3.1. Tar DNA binding protein (TARDBP) is a gene on the 1p36 locus which codes to produce a protein responsible for DNA/RNA machinery.

TARDBP accounts for ~4% of familial ALS and ~1% of sporadic ALS globally (107). TARDBP is found in up to a third of patients in Sardinia (108), suggestive of a founder effect in this region, i.e., less genetic variation in the population because it originated from a small isolated group.

1.7.4. FUS/TLS

1.7.4.1. Like TARDBP, the fused in sarcoma/translocated in liposarcoma (FUS/TLS) gene codes for DNA/RNA machinery. Mutations are found in exon 13 and lead to abnormal cytoplasmic aggregates (109). FUS/TLS accounts for ~4% of familial ALS cases but is very rare in sporadic ALS (107). It is associated with a younger age of onset, cervical site of onset, shorter survival and a lack of cognitive impairment.

1.7.5. Heritability and Genetic Pleiotropy

1.7.5.1. Twin studies estimate that the heritability of ALS is ~61% (110) while Single Nucleotide Polymorphisms (SNP) based genome wide association studies (GWAS), estimate heritability at ~21% (111). This discrepancy in heritability estimates is due to the inherent methodological differences between these two approaches (112). Twin studies show that a high proportion of ALS risk is attributable to genetic factors, while GWAS shows that these risk factors are incompletely marked by common variation in the genome. This means that the currently unknown risk genes for ALS are likely to be numerous, rare and de novo. Detailed whole genome sequencing of ALS kindred may help identify inherited risk variants.

1.7.5.2. ALS genetic risk can be conceptualised in two ways: some ALS cases are determined by a small number of genes having a large effect (e.g.,

C9orf72 or SOD1), and some ALS cases are determined by a large number of genes, each having a small individual effect, but combining to have a large cumulative effect (112).

1.8. ALS as a network disorder

1.8.1. Traditionally, ALS has been viewed as a disease of the anterior horn cells and Betz cells. A focus on neuropathology has driven concept, as do animal models of the disease (e.g., fruit flies, zebrafish and mice). However, studies of humans show that ALS can be more accurately viewed as a multi-system, network disorder (113).

1.8.2. ALS pathology begins in the pyramidal motor system which includes the motor cortex, cranial nerve motor nuclei and the spinal cord motor neurons. The pathology then spreads to neighbouring regions, particularly the prefrontal, ventral and medial frontal cortices, but also to parietal, temporal and deep grey matter structures. The spread of pathology to these regions is strongly associated with non-motor symptoms such as executive dysfunction, behavioural change and language impairment.

1.8.3. ALS pathology may spread via diffusion from one region to another, or via axonal projections to distant cerebral sites. Neuroimaging studies show degeneration of the white matter tracts that connect brain regions (46), while electrophysiological studies show increased functional connectivity between regions that is likely followed by reduced connectivity in later disease stages (47). These findings would support a network disorder model of disease spread in ALS. An fMRI study of asymptomatic ALS risk gene carriers observed a similar increase in functional connectivity between the

cerebellum and precuneus, cingulate & middle frontal lobe networks, indicating that this network dysfunction likely emerges long before first symptom onset (114).

1.8.5. The high prevalence of verbal fluency deficits in ALS suggests that the fronto-striatal circuits are likely implicated in the disease. The fronto-striatal network consists of 5 parallel neural pathways that connect frontal brain regions to the striatum, basal ganglia and thalamus (these circuits are described in more detail in Chapter 2.). Disruption of these circuits maps closely with the cognitive, behavioural and emotional changes observed in ALS. The dorsolateral prefrontal circuit is associated with executive functioning. The orbitofrontal circuit is associated with aggression, disinhibition, emotional lability and difficulty with interpersonal relationships. Damage to the anterior cingulate circuit is associated with changes to motivation.

1.9. Endophenotypes

1.9.1. Despite the genetic discoveries discussed in section 1.7., the genetic cause of ~50% of familial ALS and ~90% of sporadic ALS cases remains unknown. This may in part be because genetic studies have relied heavily on the clinical description of the disease to define the phenotype. This is problematic for two primary reasons. Firstly, ALS encompasses a wide range of genetic and neuropathological features. Secondly, the clinical phenotype is likely far removed from the underlying genetic causes of ALS. In order to address some of these issues, endophenotypes could be used to define the disease phenotype (115) (116).

1.9.2. Endophenotypes are quantitative traits which are suggested as lying between an illness and its underlying genetic cause (see figure 1.7.). They are heritable risk factors that are genetically correlated with disease liability and are measurable in both affected and unaffected individuals (117). Endophenotypes can help identify individuals at risk prior to the disease onset, and provide greater statistical power in localizing and identifying disease related genes than affection status alone (116).

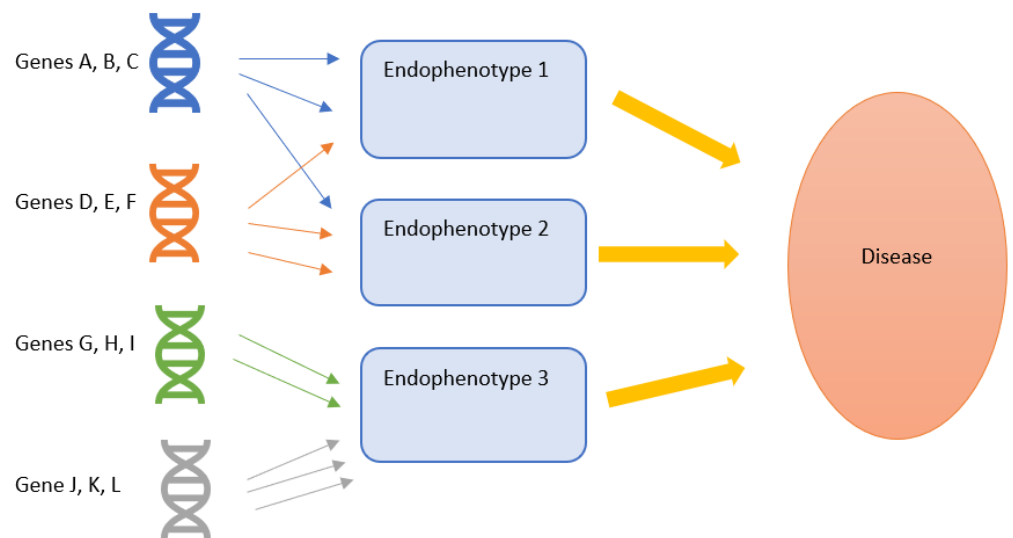


Figure 1.7. Schematic diagram representing the relationship between genes, endophenotypes and a disease.

1.9.3. The endophenotype concept originated in the field of Biology, where it was used to describe microscopic and internal traits. In contrast, exophenotypes were obvious and external, e.g., behaviour and physical appearance. Endophenotypes then became of interest in psychiatric genetics, where they were used to describe internal phenotypes which could be discoverable by a biochemical test or microscopic examination (118). The

definition of an endophenotype was later refined to refer to a measurable trait that is unobservable to the naked eye, and one that lies more proximal to the underlying genetics of a disorder (117).

1.9.4. Endophenotype research has predominantly been carried out in relation to psychiatric illness, however, there is growing awareness of their potential usefulness in understanding neurodegenerative diseases (119). For example, APOE alleles associated with Alzheimer's disease (AD) have a larger effect on AD neuropathology and cognitive outcome measures than they do on a diagnosis of AD itself (120).

1.9.5. The criteria for something to be referred to as an endophenotype has developed over time. Gottesman and Gould provide the most commonly applied criteria for endophenotypes (117). These criteria state that an endophenotype must:

- 1) Be heritable;
- 2) Be associated with the illness;
- 3) Be independent of clinical state;
- 4) Impairment must co-segregate with the illness within a family;
- 5) Found at a higher rate in nonaffected relatives than the general population.

1.9.6. Nomenclature

1.9.6.1. The term endophenotype is often used interchangeably with biomarker and intermediate phenotype despite the fact that these terms denote distinct characteristics. Biomarkers are any biological measure that is

influenced by health, illness, or an exogenous factor. Endophenotypes are considered a subset of biomarkers that are influenced by the same genetic factors that infer risk for a condition. What distinguishes endophenotypes from other classes of biomarkers are their requirement for heritability and co-segregation.

1.9.6.2. Intermediate phenotypes refer to measures indexing biological risk for a condition that are intermediate between gene expression and disease presentation. The term intermediate phenotype has also been used in the literature to describe partial dominance, leading to some confusion as to its correct use.

1.9.7. Types of Endophenotypes

1.9.7.1. Neuropsychological, neuroimaging and electrophysiological outcome measures are the most cited endophenotypes (116) (see figure 1.8.).

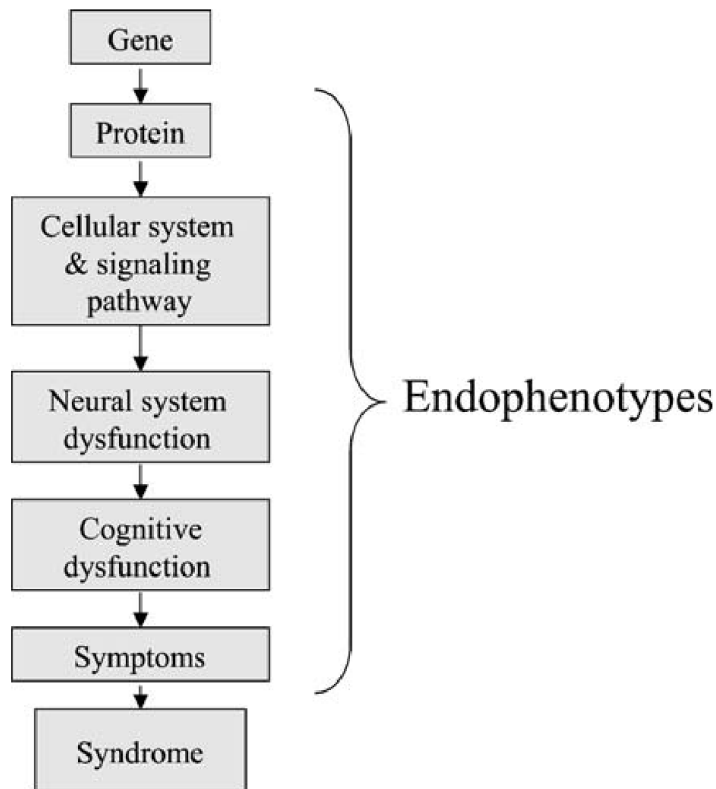


Figure 1.8. Levels of phenotypic effects, stemming from genetic factors to the disease syndrome. Endophenotypes can be found at any of the intermediate levels. Taken from Cannon and Keller (121).

1.9.7.2. Neuropsychological endophenotypes

1.9.7.2.1. Neuropsychological measures are particularly popular in

endophenotype research due to the strong heritability of cognitive ability.

Twin, family and adoption studies suggest that 60-80% of the variance in intelligence can be predicted by genetic factors (122) (123) (124). Despite its strongly genetic basis, specific genes that underpin intelligence have not been identified until recent GWA advancements, illustrating its genetic complexity. Indeed, the extent to which any trait or illness is genetically determined does not determine how simple its genetic architecture is, or how easy it is to identify relevant genes. GWA studies can now account for ~20-

50% of the heritability in intelligence, with 4% coming from polygenic risk and >10% coming from multi-polygenic risk (125).

1.9.7.2.2. Identifying genes related to general intelligence is difficult for the same reason it is difficult to identify genes related to complex diseases like ALS. Intelligence is not a one-dimensional construct, but rather is made up of numerous cognitive abilities. Consequently, studies have focused on more specific cognitive functions, such as processing speed, attention, executive functions and components of memory. These discrete abilities also have high heritability estimates and are more discrete than general intelligence, making them more promising avenues for endophenotype research.

1.9.7.3. Neuroimaging Endophenotypes

1.9.7.3.1. Neuroimaging measures are quantitative, repeatable and are more sensitive to subtle neuroanatomical changes than neuropsychological markers. This has made them another valuable avenue for endophenotype research. The heritability of neuroanatomy varies throughout the lifespan and depending on the aspect of neuroanatomy looked at. Early stages of brain development are highly influenced by genetic factors while later stages are influenced by complex gene-environment interactions (126). Heritability estimates are particularly high for whole brain grey and white matter volume (78-88%).

1.9.7.4. Electrophysiological Endophenotypes

1.9.7.4.1. Electroencephalography (EEG) has poorer structural resolution than MRI/fMRI but has much higher temporal resolution, providing an accurate measure of functional connectivity across numerous brain networks.

EEG markers such as resting state connectivity show high heritability estimates between 50-85% (127). The need for very large sample sizes potentially limits the utility of EEG or MRI/fMRI endophenotypes in gene discovery, however, they can be useful in understanding candidate genes that arise from association studies (128). Clinical information and neuropsychological/neuropsychiatric data are best suited to association studies themselves. Thereafter, EEG endophenotypes can help identify where in the brain, at what stage of the illness and the type of information processing that the gene has a role in. These latter objectives can be achieved with sample sizes that are more feasible in EEG research.

1.9.8. Critique of these mediums

1.9.8.1. One of the primary tenants of the endophenotype approach is that the genetic architecture of the endophenotype is supposedly simpler relative to the condition itself, and closer to the action of the gene. Unfortunately, neuropsychological, neuroimaging and electrophysiological endophenotypes traits tend to have complex genetic architectures themselves, which may limit their utility in gene discovery. However, an endophenotype does not necessarily need to have a simpler genetic architecture to be valuable. For example, in heart disease, low density lipoprotein cholesterol is a complex quantitative trait which is not close to gene action, but has been successfully used in identifying cardiovascular disease risk genes (129). The use of endophenotypes, in conjunction with clinical phenotyping, enrich analysis, and provide greater power for gene discovery.

1.9.9. Transcript-based Endophenotypes

1.9.9.1. Transcription is central to the expression of a gene. DNA from the nucleus is transcribed (i.e., copied) by RNA polymerase, to create microRNA (mRNA). A splice of the mRNA is then transported to the ribosome where it is translated into a protein. Gene expression techniques such as hybridisation can give a measure of the level of transcription, thus providing a measure of how active a gene is. Consequently, transcription based endophenotypes are much closer to gene action than neuropsychological, imaging or electrophysiological based endophenotypes.

1.9.9.2. Gene expression can be influenced by numerous factors such as age, sex, or even the time of day. However, genetic factors also play a significant role (130). In illnesses where variation in gene expression mediates disease risk, transcript based endophenotypes may be useful in understanding disease pathways.

1.9.9.3. Transcript-based endophenotypes have already shown promise in identifying gene pathways for Alzheimer's disease. A recent GWA study measured the expression levels of 12 late onset Alzheimer's disease (LOAD) genes and found that insulin degrading enzyme (IDE) was a risk gene for LOAD. Gene variants modified LOAD risk by mediating IDE expression (131).

1.9.9.4. Another transcript-based study identified gene expression patterns that were present in schizophrenia, Alzheimer's disease and ALS (132). All three conditions showed evidence of transcriptomic pseudoimmaturity in response to neural hyperexcitation. These findings helped identify a pool of

novel risk genes that may underlie risk for all three disorders. They also illustrate the potential of transcript-based endophenotypes in uncovering novel risk genes and elucidating the pathways from genotype to phenotype.

1.9.10. Combining traditional and transcript based endophenotypes

1.9.10.1. Whilst transcript-based endophenotypes offer significant potential for gene discovery and molecular characterisation, the relationship between them and the symptoms of a condition is often unclear. Therefore, a combination of both traditional endophenotypes (with potentially complex genetic underpinnings) and transcript-based endophenotypes (which are genetically less complex) is likely the best approach in understanding disease liability.

1.9.11. Study design in endophenotype research

1.9.11.1. In order to investigate endophenotypes, family-based study designs are necessary (133). Within the realm of family-based research, researchers can employ twin, nuclear family and/or extended pedigree study designs. Studying these populations allows for the examination of endophenotype criteria such as heritability, association to an illness and co-segregation within families. Carrying out genetic correlations can also examine the common genetic factors that influence an illness and a candidate endophenotype. Family designs must be powerful, with large sample sizes and optimal designs in order to capture subtle effects seen in unaffected relatives. To avoid false positives, researchers must control for multiple

comparisons in their analysis, a common issue in studies with a vast array of putative endophenotypes.

1.9.11.2. In order to test the requirement that endophenotypes be state independent, longitudinal paradigms would be optimal. If this is not possible, state-independence can be inferred through consensus in the literature. It is also somewhat assumed where a trait is present in unaffected relatives, and when heritability estimates are high. Studies which do not contain relatives, cannot fully establish an endophenotype. However, they can be valuable in the identification of potential endophenotypes.

1.9.12. How the literature informs this thesis

1.9.12.1. The primary objective of this thesis was to examine potential endophenotypes for ALS. To establish a trait as an endophenotype, candidate traits were evaluated based on Gottesman and Gould's criteria. A family-based research design was implemented to evaluate heritability and co-segregation. This study was limited to examining cognitive, behavioural and neuropsychiatric endophenotypes, however the findings of this research inform neuroimaging, electrophysiological and transcript-based endophenotype research, all of which can provide a separate piece of the puzzle in understanding ALS liability.

1.9.12.2. A literature review of ALS and related neuropsychiatric disorders was carried out in order to identify the most likely endophenotypes of ALS. The findings of this review are outlined in chapter 2. Chapter 3 outlines the objectives of this thesis in further detail, breaking them down into 6 specific aims.

1.9.12.3. While this thesis is primarily concerned with genetic factors that drive ALS phenotype, it also explores potential environmental factors. Cognitive reserve has been found to be protective against cognitive decline in a range of neurodegenerative disorders but has yet to be explored in ALS. Thus, the effect that cognitive reserve has on cognitive impairment in ALS was explored. As discussed in section 1.1.7., there has been contrasting findings on the nature of cognitive decline in ALS over time. These mixed findings may be due to the impact of high rates of attrition in longitudinal ALS studies. Therefore, joint modelling, a novel regression technique, was implemented to control for drop-out due to increasing disability and death, leading to less biased estimates of decline.

2. Chapter 2. Literature Review: Endophenotypes of ALS, FTD and Neuropsychiatric Disease

2.1. Introduction

2.1.1. Endophenotypes have been studied extensively in mental disorders, but rarely in neurodegeneration, particularly in rare disorders such as ALS. In this chapter, the key concepts related to endophenotype research are discussed, including heritability, aggregation and pre-symptomatic carriers. The literature on endophenotype research in ALS, FTD and related neuropsychiatric diseases is reviewed in order to identify the most promising potential endophenotypes of ALS.

2.2. Heritability

2.2.1. Heritability is a measure of the strength of genetic effects on a phenotypic trait. More specifically, it is the proportion of variance in a phenotype that can be accounted for by genetic factors (134). Mendelian genetics predicts that when a phenotype has high heritability, close relatives, such as siblings and children, are more likely to share that phenotype. Heritability is often distinguished into ‘broad sense’ heritability and ‘narrow sense’ heritability.

2.2.2. Studies of twins provide estimates of ‘broad sense’ heritability, often denoted as ‘ H^2 ’, ranging from 0 to 1. Broad sense heritability measures the proportion of phenotypic variation that is due to the total genotype, encompassing additive, dominant and epistatic components. Non-twin family studies provide estimates of ‘narrow sense’ heritability, denoted as

' h^2 '. This is a measure of the proportion of phenotypic variation that is attributable to additive genetic variance.

2.2.3. Traits determined by additive genetic variance are impacted by numerous genes, each having a small, yet cumulative impact. Traits determined by dominant inheritance are determined by few genes having a large impact. In cases where a trait is determined by epistatic variance, the presence of one gene determines the expression of another gene (operating like a switch).

2.3. Aggregation

2.3.1. Two family aggregation studies have explored the relationship between ALS and other neurodegenerative and neuropsychiatric disorders. Through detailed family histories, and verification of death certificates, an Irish aggregation study (102) found that ALS patients have family histories with higher rates of schizophrenia, psychotic illness and suicide. These findings were later replicated (135), with the additional findings of increased prevalence of autism, OCD and alcoholism in ALS kindred.

2.3.2. These studies characterised family histories prior to the onset of the patient proband to control for the confounding effect that a diagnosis of ALS could have on the mental health of a family. Higher rates of neuropsychiatric conditions were found in both C9orf72 positive and negative kindreds, suggesting pleiotropic (i.e., multiple) effects from unknown gene variants.

2.3.3. These aggregation studies found an overlap between ALS and neuropsychiatric conditions, particularly schizophrenia. Subsequent genetic

analysis validated this association, showing a significant genetic correlation between ALS and schizophrenia, estimated to be between 7-21% (136). Thus, these two conditions may share underlying neurobiological mechanisms that have yet to be explored.

2.3.4. A recent study of Scottish ALS patients found that a family history of neuropsychiatric disease appears to have a strong association with the cognitive and behavioural profile of an ALS patient. (137). For instance, a family history of mood disorders was associated with cognitive dysfunction in patients, while a family history of neurotic disorders was associated with language and visuospatial deficits. A family history of any neuropsychiatric disorder increased the likelihood of co-morbid FTD (OR = 5); as did a family history of neurotic illness (OR = 13). These findings support an earlier study of Australian ALS kindred, that found that probands with psychotic illness had an increased prevalence of mental health disorders in their relatives (138).

2.4. Pre-symptomatic ALS

2.4.1. In other neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, there is strong evidence of a long pre-symptomatic period, with pathological changes observable years, sometimes decades, before symptom onset (139) (140). Similarly, in familial ALS, there is a growing evidence of a long pre-symptomatic period, with transgenic mouse models (with SOD1 variant) showing that neuropathology is present long before overt clinical symptoms emerge (141). Motor neurons in SOD1 mice show significant alterations in their electrical properties perinatally (142),

suggesting some forms of ALS may be neurodevelopmental as well as neurodegenerative. However, SOD1 is a very rare cause of ALS, and so these mouse model findings may not be generalizable to humans.

2.4.2. A long pre-clinical and pre-symptomatic phase would represent an opportunity to better understand when and how the disease emerges and progresses; and offer a huge opportunity for novel therapeutic interventions in at-risk individuals. The gene-time-environment model of ALS proposes that ALS risk is determined by genetic predisposition which causes cell damage, and environmental factors which accumulate over time. Under this model, the clinical symptoms of ALS only emerge after years of mitochondrial dysfunction, neuroinflammation and excitotoxicity exceed a certain threshold (1). This process appears to occur in 6 sequential steps in those with no known gene mutation (143), and in just 2 - 4 steps for those with a known gene mutations like C9orf72, SOD1 or TARDBP (144). Exceeding the threshold triggers a metabolic cascade, resulting the clinical phenotype (see figure 2.1. below).

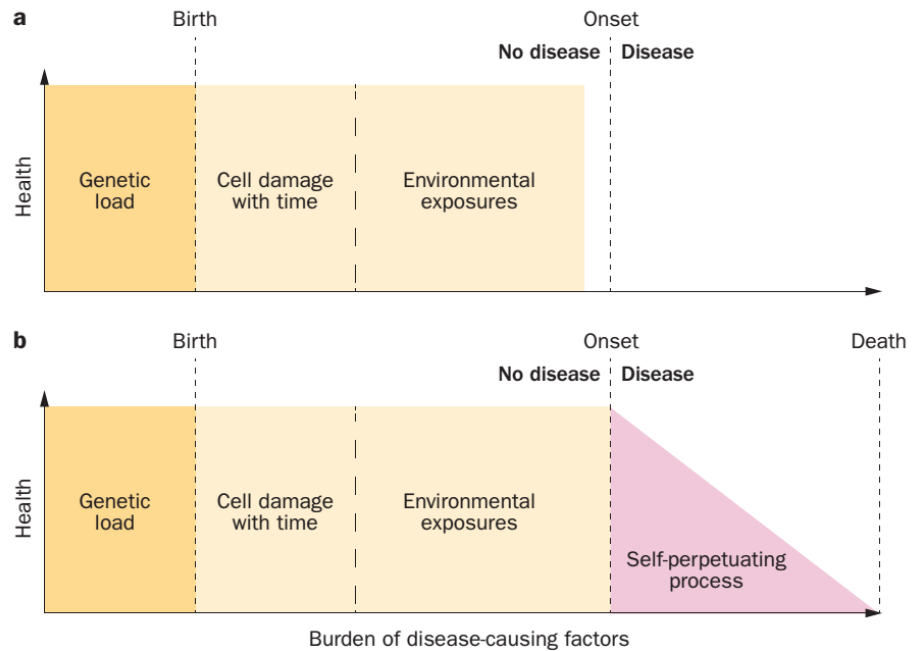


Figure 2.1. Gene-time-environment model of ALS, taken from Al-Chalabi and Hardiman (1). **a|** represents a healthy individual. At birth they have only the genetic load exists. Over time the genetic load contributes to cell damage and a person will encounter environmental exposures. The individual does not develop ALS as they do not reach the threshold. **b|** represents an individual with ALS. In this case the threshold is reached, triggering the neurodegenerative process.

2.4.3. Pre-symptomatic ALS can be distinguished into pre-manifest and prodromal stages, similar to other neurodegenerative conditions (145). The pre-manifest stage encompasses the time where biomarkers may be detected but there are no overt signs/symptoms. The prodromal stage encompasses the beginning of overt signs and symptoms right up until the disease can be diagnosed. Given the overlapping phenotypes of ALS and FTD, some individuals may be symptomatic for ALS and pre-symptomatic for FTD, and vice versa.

2.4.4. Since the discovery that a mutated C9orf72 repeat expansion was a cause of ALS, numerous studies have sought to characterize pre-

symptomatic carriers. Neuroimaging studies of pre-symptomatic carriers show progressive reductions in fractional anisotropy in the corticospinal tract (146). These studies estimate that functional and structural changes begin 12 years prior to symptom onset, although abnormalities may emerge as early as 30 years pre symptom onset (147). As gene carriers get closer to their expected age of onset they show significant reductions in cerebral blood flow in orbitofrontal, anterior cingulate and inferior parietal cortices, as well as the left middle frontal gyrus (148). In the years before a gene carrier undergoes phenoconversion (i.e., converts from pre-symptomatic to symptomatic), they show increased levels in phosphorylated neurofilament heavy chain (pNfH) in serum and corticospinal fluid (149).

2.4.5. Decades before phenoconversion, pre-symptomatic C9orf72 gene carriers show significant grey matter reduction and connectivity deficits that mimic those found in patients. As pre-symptomatic carriers get closer to phenoconversion they also begin to display evidence of cognitive changes, performing significantly worse on executive functioning and language tasks (150). Pre-symptomatic C9orf72 carriers show particular deficits on verbal fluency tasks, accompanied by distinct alterations in inferior and orbitofrontal cortices (151).

2.5. Review of endophenotypes

2.5.1. Review rationale

2.5.1.1. Given the findings of aggregation, genetic and pre-symptomatic studies, there is strong evidence of a relationship between ALS and neuropsychiatric disease. This is particularly the case for schizophrenia,

where there is a known genetic correlation between the two conditions. Aggregation studies have also highlighted suicide, OCD, autism and alcoholism as potentially related to ALS.

2.5.1.2. Consequently, a review of the literature was undertaken on endophenotypes in ALS, FTD, schizophrenia, suicide, OCD, autism and alcoholism. The aim of this review was twofold. Firstly, to explore how these seemingly independent conditions are related, and secondly to identify the best candidate endophenotypes to study in ALS. Given the significant variability in how endophenotype research has been undertaken in each field and the inconsistent use of terminology, a literature review was carried out, rather than a systematic review.

2.5.2. Search strategy

2.5.2.1. Online search engines PsychINFO, Science direct, MEDLINE and Google scholar were utilized to identify research articles on endophenotypes in ALS, FTD, suicide, OCD, alcoholism and autism. The search terms used were “Amyotrophic Lateral Sclerosis” or “Motor Neuron Disease” or “Fronto-Temporal Dementia” or “suicide*” or “Obsessive Compulsive Disorder” or “alcohol*” or “Autism”, in combination with “Endophenotype”.

2.5.3. Inclusion criteria

2.5.3.1. Studies were included if they used an observational, quantitative design. Both cross-sectional and longitudinal designs were included. Given that very few studies examined all of Gottesman and Gould’s endophenotype criteria, the review considered studies that examined at least 1 of the 5

criteria, i.e., that an endophenotype be heritable, associated with the illness, state independent, co-segregate with the illness and be present more in unaffected relatives than the general population. The review considered neuropsychological, neuroimaging and neuroelectric studies.

2.6. Results

2.6.1. ALS

2.6.1.1. The best evidence that endophenotypes of ALS exist come from pre-symptomatic studies of C9orf72 gene carriers. As mentioned in section 2.4., pre-symptomatic carriers show reduced fractional anisotropy in the corticospinal tract, reduced blood flow in orbitofrontal, anterior cingulate and inferior parietal cortices, and the left middle frontal gyrus and grey matter reduction in inferior and orbitofrontal cortices (146) (147) (148) (149). Pre-symptomatic C9orf72 carriers also show executive and language deficits, with the verbal fluency test showing the greatest sensitivity (150) (151). These neuroimaging and neuropsychological changes appear to emerge in a time dependent manner as gene carriers get closer to their expected disease onset, usually within 5 years of expected onset (150).

2.6.1.2. While the above studies suggest several candidate endophenotypes, the study designs are insufficient to test all of Gottesman and Gould's criteria, namely that the endophenotype be heritable, and co-segregate with the illness within a family. Furthermore, these studies are exclusively focused on C9orf72, which makes up only a very small proportion of all ALS cases. Direct assessment on non-C9orf72 family members is equally, if not more important than assessment of C9orf72 positive relatives, as the

identification of non-C9orf72 endophenotypes could lead to the discovery of new ALS risk genes.

2.6.2. FTD

2.6.2.1. As C9orf72 mutations are a risk factor for both ALS and FTD, the neuroimaging and neuropsychiatric traits discussed above (section 2.6.1.1.) could also be considered endophenotypes of FTD. Pre-symptomatic granulin carriers (GRN), a known risk gene for FTD, do not show any anatomical abnormalities relative to controls (152). This contrast between pre-symptomatic C9orf72 and GRN carriers suggests that ALS endophenotypes will likely be gene specific.

2.6.3. Schizophrenia

2.6.3.1. Cognitive dysfunction

2.6.3.1.1. Cognitive dysfunction is a key feature of schizophrenia, affecting quality of life and resulting in poorer functional outcomes (153). The most frequently observed cognitive impairments in individuals with schizophrenia are deficits in working memory, executive functioning, attention, language, processing speed, sensory processing and inhibition (154). However, some argue that all of these deficits are underpinned by an inability to actively represent goal information in working memory (155) (156), due to dysfunction of the dorsolateral prefrontal cortex (DLPFC) and its interaction with the thalamus, parietal cortex and the striatum, and the knock-on effect on GABA, dopamine and glutamatergic neurotransmitter systems (157).

2.6.3.1.2. Early models of schizophrenia note that unaffected relatives of schizophrenia patients have a schizophrenia-like, non-psychotic phenomenology, showing subtle thought disorders and interpersonal oddities (158). Avoidant personality disorder is significantly more frequent in unaffected relatives of schizophrenia patients, and social anxiety may be a core predisposing factor for schizophrenia (159). This suggests that genetic susceptibility to schizophrenia may manifest as a less severe schizotypal personality disorder, rather than a full schizophrenia syndrome (160).

2.6.3.1.3. Unaffected relatives of Schizophrenia patients also appear to perform significantly worse than controls on a range of cognitive tasks, such as sustained attention (161), visual working memory (162), verbal memory and inhibitory control, highlighting several potential cognitive endophenotypes of schizophrenia. Meta-analysis shows that the largest effect sizes are observed for tasks with high executive demand, such as the Trail Making Test part B test and the simple and complex continuous performance tasks (163). The deficits observed in unaffected relatives are intermediate to individuals with the clinical syndrome and the general population, i.e., unaffected relatives score worse than controls and better than patients.

2.6.3.1.4. Schizophrenia patients and their unaffected relatives also show abnormalities on numerous electrophysiological markers, such as pre-pulse inhibition, mismatch negativity and oculomotor anti-saccade, markers of sensorimotor gating, pre-attentive sensory processing and inhibitory failure (164).

2.6.3.1.5. The Consortium on the Genetics of Schizophrenia (COGS) identified executive functioning, working memory, attention, inhibition, episodic memory and social cognition as the best supported endophenotypes of schizophrenia, with clear deficits in patients and intermediate deficits in relatives (165). These various neurocognitive endophenotypes uniquely contribute to disease liability, implying multiple independently heritable endophenotypes (166).

2.6.3.2. Neurological soft signs

2.6.3.2.1. Neurological indicators are another key feature of schizophrenia, considered to reflect the contribution of genetic factors more so than environmental factors. Recently, neurological soft signs (NSS) have been studied as potential endophenotypes of schizophrenia (167). NSS consist of subtle deficits in motor co-ordination, sensory integration and motor sequencing (168). NSS are observed in ~50-65% of schizophrenia patients, compared to just ~5% of the general population (169).

2.6.3.2.2. NSS are also observable in the unaffected first-degree relatives of schizophrenia patients. Abnormal signs are most notable on the alternating fist-palm test, rapid alternating movement, go/no-go test, and fist-ring test. There is also robust evidence that NSS traits are heritable, state independent (170) and can be reliably measured (171,172).

2.6.4. Suicide

2.6.4.1. Suicide is the act of intentionally killing oneself, however, suicidality is a much more complex, multi-dimensional construct. Suicidality encompasses completed suicide, suicide attempts, suicide plans and suicidal

ideation (173). Suicidal behaviour (SB) is associated with numerous neuropsychiatric disorders, such as major depressive disorder, bipolar disorder and schizophrenia. Whilst environmental factors play a huge role in suicidality, epidemiological and genetic studies suggest that suicidality also has a significant genetic component (174). This is evidenced by the heightened prevalence of suicide in relatives of suicide victims, independent of mental illness (175).

2.6.4.2. Impulsive aggression

2.6.4.2.1. Studies of individuals who have engaged in SB, and their relatives suggest that impulsive and aggressive traits may act as endophenotypes of SB (176,177). These traits meet many of the criteria for endophenotypes in that they are reliably quantified, are highly heritable ($h^2 = \sim 40 - 47\%$), are associated with SB, are state independent and co-segregate in families with a history of SB (175,178–180).

2.6.4.2.2. High impulsive and aggressive traits in unaffected relatives have been observed in well-designed studies, including case-control, retrospective, prospective, longitudinal and family-based designs. However, these studies have traditionally relied on self-report, retrospective accounts or proxy measures from relatives to measure impulsivity and aggression, which are prone to bias and error. Future studies, utilizing neuropsychological measures of impulsive aggression may yield more defined, less biased endophenotype measures. While there is strong evidence that impulsive aggression plays a role in SB, not all SB is mediated through this process. Impulsive aggression likely reflects a subset of all SB (176).

2.6.4.3. Decision making

2.6.4.3.1. While not as well-studied as impulsive aggression, many studies have suggested that impaired decision making is an endophenotype of SB. Individuals with a history of SB show deficits on the Iowa Gambling Task, with a tendency towards disadvantageous decisions (181). Deficits on the IGT indicate an impairment in forming somatic marker, i.e., they do not generate the normal emotional response to a negative response. Impairment on the IGT is often underpinned by dysfunction of the cingulate gyrus, orbitofrontal cortex (182) and the ventromedial prefrontal cortex (183).

2.6.4.3.2. This is corroborated by post-mortem neuroimaging studies of individuals who have completed suicide, which shows atrophy in the ventral regions of the orbitofrontal cortex (184). Several studies have also found that SB is associated with a hyporeactive ectodermal response (185) (i.e., reduced palm sweat response in response to negative stimuli), implicating the ventral and medial prefrontal cortices. Electrodermal reactivity is state independent (186), ~50% heritable (187), and correlates well with the IGT (188), making it a promising endophenotype. However, further research is required in unaffected relatives.

2.6.5. OCD

2.6.5.1. Executive dysfunction

2.6.5.1.1. Individuals with OCD and their unaffected relatives show deficits in executive functioning, particularly on set shifting and response inhibition tasks (189,190). These deficits are observable when symptoms are in remission, providing good evidence that this candidate endophenotype is

state independent (191). There is also strong evidence that performance on these tasks is highly heritable (192), fulfilling another key criteria of an endophenotype.

2.6.5.2. Event related negativity

2.6.5.2.1. Models of OCD hypothesize that symptoms emerge due to dysfunction of the fronto-striatal circuitry. EEG studies have found that OCD patients and their unaffected relatives show heightened event-related negativity (ERN) (193). ERN is a sharp negative signal that accompanies an incorrect motor response. This heightened ERN signal is thought to reflect abnormal error processing in the anterior cingulate cortex (ACC) (194), a key structure in fronto-striatal circuitry.

2.6.5.2.2. ERN appears to be state independent (195) and heritable (196), making it a promising endophenotype for OCD. Diffusion Tensor Imaging (DTI) studies show white matter abnormalities in frontal and parietal regions in individuals with OCD and their unaffected relative (197). Similarly, fMRI studies show reduced activation in several frontal regions when completing a set shifting task, including abnormalities in the orbitofrontal cortex (OFC) (198).

2.6.5.3. Dysfunctional beliefs

2.6.5.3.1. Lastly, there is evidence that dysfunctional beliefs, as measured by the Obsessive Beliefs Questionnaire, is a promising endophenotype for OCD. Dysfunctional beliefs are associated with the illness, are heritable (199), and observed in unaffected relatives of OCD patients more than in the general population. When quantified, the magnitude of these dysfunctional

beliefs is less than patients, but greater than controls (i.e., it falls intermediate to the two groups) (200).

2.6.6. Alcoholism

2.6.6.1. There are several candidate endophenotypes for alcohol use disorder (AUD). The most promising of these are: subjective response to alcohol, abnormal brain oscillations and executive dysfunction. EEG endophenotypes in particular have played a crucial role in uncovering new risk genes for AUD.

2.6.6.2. EEG endophenotypes

2.6.6.2.1. Individuals with AUD and their offspring show increased power in beta frequency bands, compared to controls (201). Using a genome-wide linkage analysis of beta power in individuals at risk for AUD, researchers found a link to a GABA_A receptor gene (GABRA2) on chromosome 4 (202). Multiple studies have subsequently replicated the association between AUD and GABRA2 (203,204).

2.6.6.2.2. Other EEG studies of AUD have identified abnormal amplitude of the P300 wave ERP (Event Related Potential) in individuals with AUD and their family members (205). Abnormal P300 is particularly affected in male offspring, suggesting sex mediated effects (206). Disruption of the P300 signal suggests alterations in a person's orientation towards novel events and inhibition of cognitive processing. Linkage studies of event related oscillations that underly the P300 signal have identified a number of potential risk genes for AUD, such as the muscarinic acetylcholine receptor M2 (*CHRM2*) (207), the metabotropic 9 (*GRM8*) gene (208), the

corticotropin releasing hormone receptor 1 gene (CRHR1) (209) and the KCNJ gene (210).

2.6.6.3. Level of response

2.6.6.3.1. Level of response (LR) is the dose of alcohol required to induce a psychological effect and is distinct from alcohol tolerance. Low LR is a risk factor for AUD as an individual will require a larger quantity of alcohol to experience its effect. LR to alcohol meets many of the criteria of a candidate endophenotype for AUD (211). Individuals with a family history of AUD, show a lower LR than the general population (212), with a high heritability estimate of 67% (213). Variation in GABRA2 is associated with LR to alcohol, further validating the association between AUD and GABRA2 (214).

2.6.7. Autism

2.6.7.1. Autism spectrum disorder (ASD) has a significant genetic component, with siblings of individuals with ASD possessing a 22-fold increased risk of developing the disorder (215). However, as with many of the conditions highlighted in this review, research indicates that there are numerous genetic mechanisms that cause ASD (216), each with distinct patterns of inheritance. As a result ASD traits are likely widely and continuously distributed in both general (217) and clinical (218) populations.

2.6.7.2. The search for endophenotypes of autism is complicated by the fact that it is a neurodevelopmental disorder. Consequently, its expression, and the expression of potential endophenotypes will likely change developmentally over time (219). There is also a challenge in that there is a

wide variety of expressions of ASD, and heterogeneity in intellectual functioning. Given the significant heterogeneity of ASD, focusing on distinct endophenotypes may help identify distinct subgroups of ASD that are more closely related to the underlying genetics rather than simply the diagnosis itself.

2.6.7.3. Broader autism phenotype

2.6.7.3.1. Research suggests that ASD is associated with a broader autism phenotype (BAP), whereby unaffected relatives display a lesser variant of the condition due to shared risk genes (220). The BAP can be reliably measured using the Autism Spectrum Quotient (AQ) (221), a short self-report questionnaire of ASD traits. Mothers and fathers of children with ASD score significantly higher than controls on the AQ, suggesting that these traits are heritable and segregate with the illness. Indeed, ASD traits such as social skill deficits, communication difficulties and restrictive interests are observed in ~4-20% of first-degree relatives of people with ASD (222).

2.6.7.4. Language and social cognition

2.6.7.4.1. Due to the developmental nature of ASD, studies have examined endophenotypes that may be expressed early in life, with the goal of improving early detection. In a study by the Autism Genetic Resource Exchange (AGRE), researchers observed that children with possible ASD who speak their first words earlier show quantitative trait loci's on chromosome 3 and 7 (223). This suggests that language skills may discriminate distinct ASD subgroups in terms of their genetic liability.

2.6.7.4.2. Other potential ASD endophenotypes include theory of mind, language skills (specifically the age of first spoken word and first spoken phrase), social skills, and certain electrophysiological markers, such as asynchronization of neural activity and brain responses to emotional faces.

2.7. Discussion

2.7.1. Aggregation studies suggest a relationship between ALS and a range of neuropsychiatric disorders, including FTD, schizophrenia, suicide, OCD, alcoholism and autism. Endophenotypes may help bridge the gap between these seemingly independent disorders as they are theoretically closer to gene action. Table 2.1. highlights the most promising endophenotypes for each of these conditions. In reviewing the literature, it is clear that endophenotype research into these disorders has been considerably varied, applying different study designs and methods.

Table 2.1. Summary of candidate endophenotypes for ALS, FTD and related neuropsychiatric conditions.

Condition	Cognitive Endophenotypes	Neuroimaging Endophenotypes	EEG Endophenotypes	Other endophenotypes
ALS - FTD	Executive dysfunction, Language dysfunction, Verbal fluency deficits	Abnormalities in corticospinal tract, orbitofrontal, anterior cingulate and inferior parietal cortices, and left middle frontal gyrus	-	-

Schizophrenia	Working memory, executive functioning, attention, language, processing speed, sensory processing and inhibition deficits	-	Pre-pulse inhibition, mismatch negativity	Neurological soft signs
Suicide	Decision making impairment	Atrophy in the orbitofrontal cortex	-	Impulsive aggression, hyporeactive ectodermal response
OCD	Set shifting and response inhibition deficits	White matter abnormalities in frontal and parietal regions, reduced activity in the orbitofrontal cortex	Heightened event related negativity	Dysfunctional beliefs
Alcoholism	Executive dysfunction	-	Increased power in beta bands, abnormal amplitude of the P300 wave ERP	Low level of response to alcohol
Autism	Theory of mind deficits, language abnormalities	-	Asynchrony of brain activity in response to emotional faces	Broader autism phenotype, Age of first words

2.7.2. One commonality observed across these multiple conditions is that they, and their accompanying endophenotypes, are often associated with disruption to the fronto-striatal circuits. This is supported by neuroimaging, electrophysiology and neuropsychological studies of patients and unaffected relatives. The fronto-striatal circuits consist of 5 parallel neural pathways that connect frontal brain regions to the striatum, basal ganglia and thalamus. A motor circuit, originating in the supplementary motor area; an oculomotor circuit stemming from the frontal eye fields; and 3 circuits stemming from the prefrontal cortex: the dorsolateral prefrontal, anterior cingulate and orbitofrontal circuits (224).

2.7.3. These 5 circuits can be divided into 3 functionally distinct groups, sensorimotor, associative and limbic circuits (see figure 2.2.). Sensorimotor circuits mediate motor control and habitual instrument behaviour, associative circuits mediate executive functioning and goal-mediated behaviour, and limbic circuits mediate emotional processing and reinforcement learning (225). Each circuit originates in the frontal lobes, then projects to the striatum via the globus pallidus, the putamen, the substantia nigra and the thalamus. The thalamus connects back to frontal regions creating a closed feedback loop.

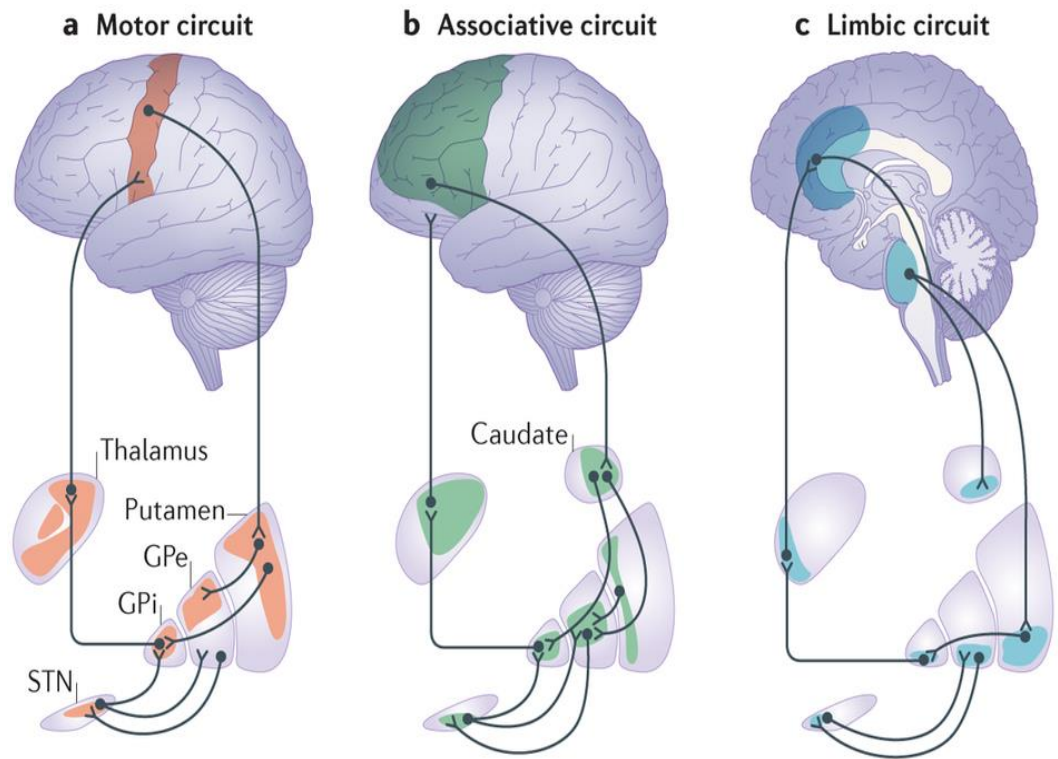


Figure 2.2. The functional divisions of the sensorimotor, associative and limbic fronto-striatal circuits. Taken from Jahanshahi et al. (225). GPe - external segment of the Globus Pallidus; GPi – internal segment of the Globus Pallidus; STN – Subthalamic Nucleus

2.7.3. Dorsolateral prefrontal circuit

2.7.3.1. The dorsolateral prefrontal circuit is primarily involved in executive functions, such as problem solving, planning and the sequencing of events (224). Many of the cognitive deficits observed in ALS, such as executive impairment and verbal fluency deficits suggest an underlying disruption to the supervisory attention system (226), which is mediated by the dorsolateral prefrontal cortex.

2.7.4. Orbitofrontal circuit

2.7.4.1. The orbitofrontal circuit connects frontal regions to the limbic system. Damage to this circuit often results in personality changes, such as

aggressiveness, disinhibition, emotional lability and difficulty with interpersonal relationships (227). ALS patients with and without co-morbid FTD will often show these symptoms, with social cognition deficits now recognized as a key feature of ALSci (228).

2.7.5. Anterior cingulate circuit

2.7.5.1. The anterior cingulate circuit is involved in motivated behaviour, monitoring, working memory and novelty detection. Dysfunction of this circuit will often result in apathy. In ALS, apathy is strikingly common, with moderate to severe apathy present in up to 41% of ALS patients (55).

2.7.6. Fronto-striatal dysfunction across ALS and neuropsychiatric disease

2.7.6.1. The literature highlighted in this review suggests that fronto-striatal circuits are implicated in ALS, FTD, and related neuropsychiatric conditions. Structural MRI shows that ALS patients have significant atrophy in the basal ganglia, including the caudate nucleus, hippocampus and nucleus accumbens (229). Atrophy in these regions results in dysfunction of fronto-striatal circuits, accounting for the neuropsychological profile in ALS, namely executive dysfunction, apathy and social cognitive deficits (230). Basal ganglia pathology is more extensive, but not solely found in C9orf72 patients, suggesting that this gene is one of many to influence fronto-striatal circuits.

2.7.6.2. Neuroimaging and neuropathological findings show significant atrophy of the striatum in Fronto Temporal Lobar Degeneration (FTLD), with differential involvement according to FTLD subtype (231). There is

evidence of a gradient of striatal atrophy according to FTLN subtype.

Behavioural variant FTD (bvFTD) shows the greatest degree of striatal atrophy, followed by FTD, then progressive non-fluent aphasia and finally semantic dementia (232). Striatum involvement occurs early in the disease, making it a promising biomarker of FTLN. Disruption to the associative and limbic fronto-striatal networks reflects the neuropsychological profile of FTLN patients, namely apathy, impulsiveness and disinhibition.

2.7.6.3. Fronto-striatal circuits are also implicated in schizophrenia.

Schizophrenia patients show dysfunction of the limbic circuits early in the disease but not the sensorimotor or associative ones (233). Unaffected relatives also show disruption to the limbic circuit, specifically in relation to reward processing (234). Functional MRI studies show that unaffected relatives have decreased activation of the ventral striatum in anticipation of reward and increased activation when receiving the reward.

2.7.6.4. Neuroimaging studies have consistently shown associations between suicidal behaviour and fronto-striatal dysfunction (235). Numerous reviews agree that there is specific involvement of the dorsolateral and orbitofrontal prefrontal cortex, key regions of fronto-striatal circuitry. Grey and white matter hyperintensities are 5-8 times more likely in patients with suicide attempts compared to those without attempts (236). However, it remains unclear whether these exist prior to the suicide attempt, or are a consequence of the suicide attempt, e.g., anoxia due to hanging. Neuroimaging of individuals with a family history of suicide suggests that such fronto-striatal dysfunction is in some part inherited. Individuals with a family history of

suicide show reduced volumes in temporal regions of the right dorsolateral prefrontal cortex and left putamen (237). Suicide by violent means was associated with increased caudate volume, suggesting that dysfunction of certain fronto-striatal circuits infers risk for suicidality, while dysfunction of others modulates the type of suicidal behaviour.

2.7.6.5. Fronto-striatal dysfunction is well documented in OCD. Both patients with OCD and their first-degree relatives show hypoactivation of the right dorsolateral prefrontal cortex during goal directed tasks (238). They also show reduced functional activity between the dorsolateral prefrontal cortex and the basal ganglia. This provides good evidence that fronto-striatal dysfunction is a heritable endophenotype for OCD.

2.7.6.6. The fronto-striatal system is strongly implicated in inhibitory processes. Damage to these circuits, and the subsequent impairment to inhibition is often associated with alcohol use disorder. Individuals with more severe alcohol dependence show less connectivity between frontal regions and the striatum (239). Altered connectivity is also observed in unaffected relatives and those at high risk for alcohol use disorder (240). Longitudinal studies of adolescents at risk for alcoholism have identified alterations in white matter structures prior to any alcohol consumption, which predicts those who go on to develop alcohol use disorder in later life (241).

2.7.6.7. Lastly, fronto-striatal dysfunction is hypothesized to contribute to key symptoms of ASD, namely social interaction and communication difficulties, and restricted interests and repetitive behaviours. In contrast to

many conditions characterized by reduced connectivity, ASD is associated with increased functional connectivity in fronto-striatal regions, such as the anterior cingulate cortex, the orbitofrontal cortex and the striatum (242). Heightened connectivity between the middle frontal gyrus and the caudate is associated with more repetitive behaviours, while increased connectivity between the paracingulate gyrus and the orbitofrontal gyrus to the nucleus accumbens is associated with social interaction and communication deficits (242). Similar structural and functional abnormalities in the fronto-striatal circuits are also observed in parents of individuals with ASD (243). The neural pattern observed in parents appears to be intermediate between individuals with ASD and healthy controls (243). There is also a positive association between the degree of fronto-striatal dysfunction and the degree of autistic traits as measured by the AQ.

2.8. How the literature informs this thesis

2.8.1. This literature review elucidates the association between ALS, FTD and the neuropsychiatric conditions that are highly prevalent in ALS kindred. The various conditions explored all implicate disruption to the fronto-striatal circuits. However, the conditions vary somewhat in which networks are involved. For example, schizophrenia is associated with more limbic network dysfunction; ALS and OCD are associated with dorsolateral prefrontal and anterior cingulate network dysfunction; and FTD, autism and suicidality are associated with greater orbitofrontal circuit dysfunction.

2.8.2. Involvement of the fronto-striatal circuits has significant implications for this thesis. While there are no known endophenotypes for ALS, the

literature suggests that the most likely candidate traits will be related to fronto-striatal functioning. Consequently, the methodology applied in this thesis endeavoured to explore the functions of these networks. Specifically, the neuropsychological battery of tests that was implemented include tests of each network. The dorsolateral prefrontal cortex networks were evaluated using numerous executive functioning tests, such as the colour-word interference test, the digit span test and the FAS verbal fluency task. Orbitofrontal circuits were explored using the reading the mind in the eyes test and the Iowa Gambling Test. The anterior cingulate circuit was examined using informant reported behavioural questionnaires, such as the Beaumont Behavioural Inventory and the Frontal Systems Behaviour Scale.

2.8.3. In examining these functions in a sample of unaffected relatives of ALS patients, this thesis builds on aggregation, pre-symptomatic and heritability studies. Aggregation studies have relied on family history questionnaires. In this study participants were given a neuropsychiatric traits questionnaire to explore the extent to which ALS relatives report psychotic, OCD, AUD, Autism and suicidality traits. The direct assessment of relatives of ALS patients was designed to uncover the true extent to which relatives present with neuropsychiatric or neuropsychological traits.

2.8.4. Assessment of ALS patients and their unaffected relatives enabled examination of the heritability of any neuropsychological and neuropsychiatric endophenotypes identified. Testing for the presence of the C9orf72 repeat expansion in this project enabled the capture of a significant amount of pre-symptomatic C9orf72 carriers. Sub-group analysis of these

participants builds on the many international studies of pre-symptomatic C9orf72 carriers, providing the first study of this population in Ireland.

3. Chapter 3. Aims

3.1. Introduction

3.1.1. ALS is characterized by a wide range of clinical, cognitive and genetic profiles. While some individuals with ALS will remain cognitively unchanged throughout their illness, others will experience cognitive decline or behavioural change. The cognitive functions most typically effected in individuals with ALS are executive and language domains, which are underpinned by disruption to fronto-striatal circuits.

3.1.2. Aggregation studies have indicated that family members of ALS patients have heightened risk of psychosis, suicide, alcoholism, OCD and autism. While these syndromes may appear rather distinct, they are all associated with disruption to varying components of fronto-striatal circuits.

3.1.3. In investigating potential endophenotypes for ALS, it was hypothesized that relatives of ALS patients experience disruption to fronto-striatal circuits, like that of patients, due to a shared genetic risk. This may manifest cognitively, as measured by tasks of executive functioning, or psychiatrically, as shown by measures of psychosis, OCD, suicidality, alcoholism or autism traits.

3.1.4. Endophenotypes may help in the identification of new ALS related risk genes and help in understanding the biological pathways from genotype to phenotype. When considering cognitive impairment in ALS, one potentially moderating variable is cognitive reserve. Cognitive reserve is a theoretical concept, used to account for the mismatch between brain

pathology and overt symptomatology. Individuals with high cognitive reserve appear to be resilient against the effect of brain pathology in conditions like Alzheimer's, FTD, Parkinson's and Huntington's disease.

3.2. Primary objectives

3.2.1. This thesis was broken down into 6 distinct aims. Aim 1 addressed how cognition was reliably measured in ALS. Aim 2 of the thesis explored longitudinal cognitive decline in ALS, and how lifestyle factors protect against decline. Aims 3, 4, 5 and 6 then addressed the primary objective of the thesis, which was to examine potential endophenotypes of ALS. Aim 3 explored potential cognitive endophenotypes, aim 4 examined neuropsychiatric endophenotypes, aim 5 and 6 addressed how endophenotypes relate and/or delineate from one other.

3.3. Aims

3.3.1. Aim 1

3.3.1.1. Aim 1 of this thesis was to examine the equivalency and practice effects of Edinburgh Cognitive and Behavioural ALS screen (ECAS) alternative versions. This enabled the reliable measurement of cognitive decline in ALS over time required for aim 2. In testing this aim, 2 specific hypotheses were proposed.

Hypothesis 1: ECAS versions A, B and C are equivalent, as found by a similar study in a Scottish population (34).

Hypothesis 2: Serial assessment of the ECAS, using alternate versions A, B and C does not produce practice effects.

3.3.2. Aim 2

3.3.2.1. Aim 2 of this thesis was to examine the effect of cognitive reserve in ALS. This examined the nature of longitudinal cognitive change in ALS and potentially modifiable factors. In order to achieve this aim, one specific hypothesis was proposed.

Hypothesis 1: Cognitive reserve has a significant moderating effect on cognitive change over time.

3.3.3. Aim 3

3.3.3.1. Aim 3 of the thesis was to compare the cognitive profile of relatives of ALS patients and healthy controls. Relatives of ALS patients and healthy controls were compared on an extensive battery of neuropsychological tests, including measures of intelligence, executive functioning, language, memory and attention (see chapter 4 for full description). In completing this aim, 3 specific hypotheses were proposed.

Hypothesis 1: Relatives of ALS patients perform significantly worse than controls on executive functioning and language tasks, representative of disruption to fronto-striatal circuits.

Hypothesis 2: Relatives of familial ALS patients perform significantly worse than relatives of sporadic ALS patients on executive functioning and language tasks, due to the stronger genetic factors present in these individuals.

Hypothesis 3: Relatives of ALS patients that are asymptomatic C9orf72 gene carriers perform significantly worse on executive functioning tasks than

relatives who are C9orf72 negative, as found in previous asymptomatic studies.

3.3.4. Aim 4

3.3.4.1. Aim 4 of the thesis was to compare neuropsychiatric traits in relatives of ALS patients and healthy controls. Relatives of ALS patients and healthy controls were compared on a range of neuropsychiatric traits questionnaires, including measures of depression, anxiety, impulsiveness, OCD, ADHD, autism, psychosis and personality. In completing this aim, 3 specific hypotheses were proposed.

Hypothesis 1: Relatives of ALS patients score significantly higher than controls on measures of psychosis and autism traits, representative of disruption to fronto-striatal functioning.

Hypothesis 2: Relatives of familial ALS patients score significantly higher than relatives of sporadic ALS patients on measures of psychosis and autism traits, due to stronger genetic factors present in these individuals.

Hypothesis 3: Asymptomatic C9orf72 gene carriers score significantly higher than C9orf72 negative individuals on psychosis traits, indicative of the known genetic association between C9orf72 and schizophrenia.

3.3.5. Aim 5

3.3.5.1. Aim 5 of this thesis was to examine the effect of IQ on neuropsychological endophenotypes. In testing this aim, 1 specific hypothesis was proposed.

Hypothesis 1: Cognitive endophenotypes are not attributable to deficits in IQ.

3.3.6. Aim 6

3.3.6.1. Aim 6 of this thesis was to examine how cognitive and neuropsychiatric endophenotypes relate to each other. In testing this aim, 1 specific hypothesis was proposed.

Hypothesis 1: Cognitive and psychiatric endophenotypes exist in distinct clusters, representative of various ALS sub-phenotypes.

4. Chapter 4. Equivalency and Practice Effects of ECAS

Versions A-B-C

Published Work List

The work described in chapter 4 has been published in the peer reviewed journal ‘Amyotrophic Lateral Sclerosis and Frontotemporal Dementia’ as:

Costello E, Lonergan K, Madden C, O’Sullivan M, Mays I, Heverin M, Pinto-Grau M, Hardiman O, Pender N. Equivalency and practice effects of alternative versions of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2020 Jan 2;21(1-2):86-91.

4.1. Introduction

4.1.1. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) is a brief, yet sensitive screening tool in ALS (33). It has been translated into multiple languages (244–246) and has been validated against a full neuropsychological battery (247,248). In addition to detecting cognitive symptoms when they arise, it is equally important to track cognitive changes over time. Alternative versions of the ECAS (ECAS-B and ECAS-C) were developed specifically for this purpose (34). These new versions follow the same structure as ECAS A but utilize new stimuli, e.g., new pictures for item naming, a new story for memory recall, etc.

4.1.2. In order to reliably measure clinically meaningful change from one time point to another, neuropsychologists will often utilize reliable change index (RCI) scores. RCI formulae generally take the form of a measure of true change as the numerator and the corresponding standard error as the denominator (249). An RCI approach is advantageous as it minimizes measurement error, can account for practice effects and is easy to interpret. RCI has been utilized to signify reliable change in a range of conditions such as schizophrenia, epilepsy, concussion and dementia (250–253). An initial study found that serial administration of alternative forms of the ECAS (i.e., ECAS A-B-C) does not produce practice effects and reliable change index (RCI) scores have been proposed for a Scottish population (35). For example, on ECAS total score (which ranges from 0 – 136), a decline of 9 points would suggest clinically meaningful decline).

4.1.3. The purpose of this study was to examine if ECAS versions A, B and C can be considered equivalent, and to generate normative data from an Irish population. This study also investigated if serial administration of the ECAS produces practice effects in an Irish cohort and proposes RCI scores to help interpret cognitive change over time. It was hypothesized that alternative versions would be equivalent, and not produce practice effects.

4.2. Method

4.2.1. Participants

4.2.1.1. Healthy Irish controls were recruited for two studies, one to assess the equivalency of ECAS versions A, B and C (study 1), and another to examine practice effects of serial administration (study 2). The first cohort of 176 participants completed either ECAS A, B or C at one time-point only. The second cohort of 60 participants underwent serial ECAS assessments, completing version A first, then B, then C. Exclusion criteria included a history of intellectual or learning disability, the presence of a mood disorder or psychiatric disorder, neurological conditions affecting cognition and/or a family history of ALS. All participants spoke English as their first language. Informed written consent was obtained from all participants.

4.2.2. Procedure

4.2.2.1. In study 1, participants (n = 176) were administered either ECAS A, B or C only. In study 2 (n = 60), participants were administered ECAS A-B-C serially, approximately 4 months apart (median was 4.2 months between T1 and T2, and 4.3 months between T2 and T3). Participants were tested in their own home, or if preferred, in a quiet room in Trinity Biomedical Science Institute, Dublin. Verbal fluency index (vfi) converted scores were generated using procedures outlined on the ECAS website: '<https://ecas.psy.ed.ac.uk/>' and are presented in supplemental table 1. The ECAS was administered by the thesis author 'EC' and other assistant psychologists or research assistants in the Beaumont Hospital psychology department. All testers were trained using the ECAS training guide at '<https://ecas.psy.ed.ac.uk/>'. To ensure testing and

scoring were consistent, multiple participants were scored by multiple testers, and intra class correlations (ICC) were carried out. Once an ICC higher than .8 was achieved a tester could test on their own.

4.2.3. Data analysis

4.2.3.1. Statistical analysis was carried out using SPSS (Version 25) and R (Version 3.5.1). Demographic information of each group was compared using chi square tests and one-way analysis of variance (ANOVA). Shapiro-Wilk test, histograms and normality plots were used to determine if data was parametric. Log transformations were performed for data which violated statistical assumptions. Where transformation failed to correct for violations, non-parametric tests were applied. To control for multiple comparisons, the Benjamini-Hochberg (254) procedure was implemented, using a false discovery rate of 5%. Power was calculated using G*power software (255).

4.2.3.2. Study 1: To investigate equivalency, one-way analyses of covariance (ANCOVA) were performed to compare ECAS total scores, composite scores and sub-scores across groups. To detect a small effect size ($d = 0.3$), with a power of .8, a minimum sample size of 111 was required. Age and education were added as covariates due to the significant relationships between these variables and ECAS performance (see supplemental table 2). Kruskal-Wallis (K-W) tests were performed where data violated assumptions of parametric analysis. Two one sided t-tests (TOST) were performed to directly test equivalency between ECAS groups. Unlike traditional tests, this procedure assumes that there is a difference between groups, specifying upper and lower limits that are considered meaningful (e.g., $d = -0.3$ to $+0.3$ to test a small

effect). T-tests are then carried out on each limit. If the difference between groups falls within these limits, the groups are deemed equivalent (256).

4.2.3.3. For this TOST procedure, the pre-determined minimum meaningful difference between ECAS versions was a Cohens $d = -0.5$ to $+0.5$ as upper and lower limits respectively. An alpha level of 0.1 was applied (two tailed), giving a power of 0.8.

4.2.3.4. *Study 2:* To investigate practice effects, repeated measures ANOVAs were carried out on ECAS total scores, composite scores and sub-scores across each time point. In cases where Sphericity was violated, Greenhouse-Geisser, Huynh-Feldt or Lower bound corrections were applied. Post-hoc Sidak tests were used to compare sub-groups if a significant main effect was observed. To detect a small effect size ($d = 0.15$) and achieve a power of .8, a minimum sample size of 31 was required.

4.2.3.5. Test-retest reliability was assessed using intra-class correlation coefficients (ICC) with mean-rating absolute agreement two-way effects models. To provide clinically meaningful thresholds of cognitive decline, reliable change index (RCI) scores were calculated. The Chelune method (257) of calculating RCI was applied. This approach takes into consideration the impact of practice effects and measurement error. An alternative standard error of difference (SE_{diff}), proposed by Iverson (258), was applied. This calculation of the standard difference is more appropriate as it considers variability in Time 2 scores. To calculate RCI scores, the following equation was utilized:

$$\Delta X = (\bar{X}_2 - \bar{X}_1) \pm 1.645 (SE_{diff})$$

where $SE_{diff} = \sqrt{SEM_1^2 + SEM_2^2}$, $SEM_1 = s^1 \sqrt{1 - r_{xx}}$, SEM is the standard error of measurement, s^1 is the standard deviation of the ECAS version being compared against (e.g., when comparing ECAS A and B, s^1 is the standard deviation of ECAS A), and r_{xx} is the test re-test correlation coefficient.

4.3. Results

4.3.1. Study 1: Equivalency of ECAS versions

4.3.1.1. One-way ANOVAs and chi-square tests showed no significant difference between ECAS groups in terms of age, education and gender (see table 4.1.).

Table 4.1. Mean (standard deviation) age, years of education and gender frequency of participants who completed ECAS A, B or C only.

	ECAS A (n = 70)	ECAS B (n = 52)	ECAS C (n = 54)	F/ χ^2 (df)	p-value
Age	62.70 (8.65)	63.65 (10.27)	65.94 (11.09)	1.67	.19
Years of Education	15.53 (4.74)	16.79 (3.63)	15.55 (3.26)	1.77	.17
Gender	27F/43M	29F/23M	29F/25M	4.45	.11

4.3.1.2. ANCOVA and K-W tests were carried out to compare mean ECAS performance across each ECAS versions. No significant differences were observed between groups on all sub-scores, except for the visuospatial sub-test, where participants scored significantly worse on version C, compared to ECAS A and B (see table 4.2.).

Table 4.2. Mean (standard deviation), ANCOVA F value/K-W chi square and p-value comparing ECAS A, B and C.

Domain (max score)	ECAS A (n = 70)	ECAS B (n = 52)	ECAS C (n = 54)	F/ χ^2 (df)	p
ECAS Total (136)	114.16 (11.73)	115.85 (9.54)	113.07 (11.97)	.34 (2,171)	.71
ALS Specific (100)	85.07 (8.85)	86.27 (7.34)	83.85 (9.41)	.49 (2,171)	.62
Language (28)	26.84 (1.66)	26.9 (1.76)	26.48 (1.79)	3.54 ^k (2, n = 176)	.17
Verbal Fluency (24)	19.71 (2.32)	19.69 (2.67)	18.93 (4.3)	.90 (2,171)	.41
Executive (48)	38.51 (6.42)	39.67 (5.01)	38.44 (6.02)	.47 (2,171)	.63
ALS Non-Specific (36)	29.09 (4.62)	29.58 (3.87)	29.22 (3.85)	.30 (2,171)	.75
Memory (24)	17.5 (4.3)	17.96 (3.67)	18.15 (3.31)	.97 (2,171)	.38
Visuospatial (12)	11.59 (.83)	11.62 (.63)	11.07 (1.03)	15.08 ^k (2, n = 176)	.001*

k – Assumptions of ANCOVA were violated, Kruskal-Wallis test used

* Statistically significant after controlling for multiple comparison

4.3.1.3. To directly test the equivalency of ECAS versions, the two one sided t-test (TOST) procedure was applied (see table 4.3.). ECAS versions A and B were equivalent on all ECAS domains. ECAS A and C were equivalent on all ECAS domains except for visuospatial functioning. ECAS B and C were not equivalent for ECAS total, ALS specific score or visuospatial functioning.

Figure 4.1. below illustrates the ECAS total TOST results. Note that while ECAS B and C are not statistically equivalent, the difference between them is minimal.

Table 4.3. TOST procedure t value (df) and p-values comparing ECAS A-B, A-C and B-C (n = 60).

	ECAS A-B TOST		ECAS A-C TOST		ECAS B-C TOST	
	T (df)	p	T (df)	p	T (df)	p
ECAS Total	1.89 (119)	.03	-2.25 (113)	.01	-1.25 (100)	.11*
ALS Specific	1.96 (119)	.03	-2.02 (111)	.02	-1.10 (100)	.14*
Language	2.53 (106)	.006	-1.60 (110)	.06	-1.36 (104)	.09
Verbal Fluency	-2.66 (100)	.005	-1.46 (76)	.07	-1.49 (89)	.07
Executive	1.66 (120)	.05	-2.71 (117)	.004	-1.43 (102)	.08
ALS Non-Specific	2.53 (106)	.02	-1.60 (109)	.005	-1.36 (104)	.02
Memory	2.13 (118)	.02	1.86 (122)	.03	2.29 (102)	.01
Visuospatial	2.56 (120)	.005	.31 (100)	.62*	.75 (88)	.77*

* Not statistically equivalent

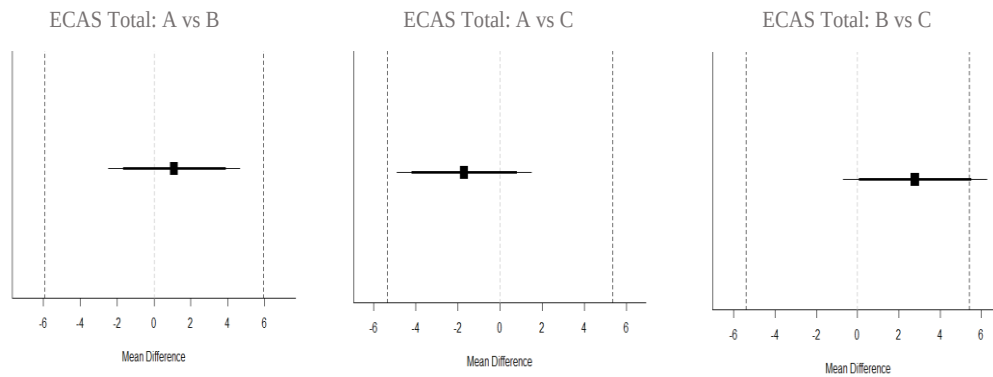


Figure 4.1. TOST results, displaying mean differences between ECAS A, B and C total score.

4.3.2. Study 2: Practice effects of serial assessment

4.3.2.1. An independent cohort of participants completed serial ECAS assessment (See table 4.4. for descriptives).

Table 4.4. Mean (standard deviation) and range of age, years of education and gender frequency of participants who completed ECAS A - B - C serially.

	ECAS A-B-C (n = 60)	
	Mean (SD)	Range
Age	64 (7.36)	38-80
Years of Education	15.42 (4.82)	8-40
Gender	39 M/ 21 F	

4.3.2.2. Repeated measures ANOVAs were carried out to compare ECAS scores across each time point (see table 4.5.). Significant effects for time were observed for ECAS Total, ALS specific and ALS non-specific composite scores (See figure 4.2.). Significant effects for time were also observed for verbal fluency and memory sub-scores. Post-hoc Sidak tests revealed that

verbal fluency significantly improved from version A to B, while memory scores significantly improved from ECAS B to C.

Table 4.5. Mean ECAS score, ANOVA p-value, and post hoc Sidak p-value for serial ECAS assessment (n = 60).

	ECAS A	ECAS B	ECAS C	F (df)	A-B MD	B-C MD
ECAS Total	109.2 (16.1)	112.7 (15)	112.3 (16.5)	10.16* (2, 166)	-.21	-.14
ALS Specific	81 (13.1)	83.8 (11.9)	82.4 (13.4)	7.49* (2, 118)	-.21	.08
Language	26.0 (3.2)	25.8 (3)	25.7 (3.3)	2.49 (2, 122)	-	-
Verbal Fluency	18.5 (3.9)	19.9 (3.8)	19.2 (5.1)	11.40* (2, 120)	.42*	-.13
Executive	36.4 (7.4)	38.1 (6.9)	37.8 (6.9)	2.52 (2,124)	-	-
ALS Non-Specific	28.3 (4.6)	29 (4.2)	30 (7.4)	8.10* (2, 124)	.12	.16
Memory	16.8 (4.4)	17.8 (3.6)	18.5 (7.2)	11.57* (2,124)	.16	.17*
Visuospatial	11.5 (1)	11.2 (1.2)	11.2 (1)	.41 (2, 124)	-	-

* Statistically significant after controlling for multiple comparison; MD – Mean Difference

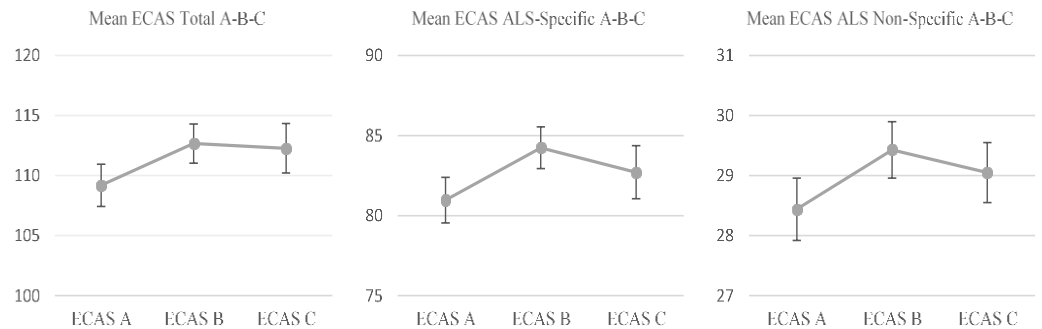


Figure 4.2. Mean ECAS total score on serial A-B-C assessment.

4.3.2.3. Test-retest reliability: Intra-class correlation coefficients (ICC) were calculated for ECAS total, ALS specific and ALS non-specific scores (see supplemental table 3.). All ECAS versions showed excellent test-retest reliability, with all coefficients higher than 0.7.

4.3.2.4. Reliable change index (RCI) scores were calculated using the Chelune method. Based on these calculations, cut-offs are presented as thresholds for determining clinically meaningful cognitive decline (See table 4.5.).

Table 4.6. Reliable Change Index (RCI) scores for ECAS A, B and C.

	ECAS total	ALS Specific	ALS Non-Specific
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

4.4. Discussion

4.4.1. The results of this study suggest that alternative ECAS versions A, B and C are comparable, but not strictly equivalent. A significant difference was observed on the visuospatial sub-task, in line with previous findings (35). TOST analysis found that ECAS A was equivalent to ECAS B and C, however, ECAS B was not equivalent to ECAS C. Population derived, version-specific norms and RCI scores are recommended to determine abnormal performance. Irish specific norms (see supplemental tables 4 and 5) and RCI cut-offs are provided to aid this approach.

4.4.2. Contrary to previous research (35), this study found evidence of practice effects on the ECAS over time. Participants significantly improved from time one to time two, particularly on the verbal fluency task. Participants did not improve on ECAS total from time 2 to time 3, however, there was significant improvement on the memory subtask. Practice effects in neuropsychological batteries are most common on novel tasks where participants can learn

advantageous test strategies (259). This may account for the slight improvement in verbal fluency. Previous studies have also shown that the largest improvements are usually from time 1 to time 2, and then reduced thereafter (260), as was found here.

4.4.3. Improvement over time may be due to participants becoming increasingly comfortable with testing conditions or through improved task learning strategies (261). While alternative forms do not completely remove the presence of practice effects, they do greatly reduce them. Repeated administration of ECAS A (A-A-A) produces much larger practice effects (262) than those found here using serial assessment (A-B-C). This study retested individuals in intervals of 4 months, in line with previous research (35,262). Longer intervals may reduce the presence of practice effects, however further study is required. Future research is also needed to examine the effects of alternative ECAS sequences, e.g., C-A-B, B-C-A, etc.

4.4.3. In order to provide clinicians with a practical means of identifying cognitive change over time, while accounting for potential practice effects, Irish specific RCI cut-offs are presented. Decline or improvement greater than these thresholds can be considered indicative of clinically meaningful change. For example, decline greater than 10 from ECAS A to B, can be considered clinically meaningful and warrant further investigation. These thresholds compliment previously proposed cut-offs by Crockford et al. (35), who proposed a decline of 9 on ECAS total as an indicator of clinically meaningful change in a Scottish cohort. The publication of Irish specific cut-offs and normative data add to the utility of the ECAS as a screening measure across

various countries, allowing reliable comparison between patients from different countries.

4.4.4. While RCI scores will give greater clarity to those applying the ECAS, it is essential to bear in mind the limitations of this approach. As with any unidimensional metric of abnormality, RCI thresholds fail to account for individual differences. Clinicians must be vigilant that age, education, baseline performance, current distress and differential practice effects are unique to each individual (263).

4.4.5. This study is limited by the relatively narrow range of age and education of participants and by the fact that anxiety and depressive were not measured. Previous research has shown the ECAS to be highly sensitive and specific in identifying cognitive impairment. However, it is important to acknowledge its intended use is as a screening tool and, when possible, should be followed up by a full neuropsychological assessment by a trained neuropsychologist. Given the contrasting findings between this study, and previous literature, further research is needed to clarify the psychometric properties of alternative ECAS versions, and the extent to which practice effects occur. In doing so, the ECAS will become an increasingly useful tool in understanding the nature of cognitive and behavioural deficits in ALS, informing clinicians in everyday care, and providing a useful metric for clinical trials.

5. Chapter 5. The Association between Cognitive Reserve and Cognitive Decline in ALS

Published Work List

The work described in chapter 5 has been published in the peer reviewed journal 'Journal of Neurology, Neurosurgery and Psychiatry' as:

Costello E, Rooney J, Pinto-Grau M, Burke T, Elamin M, Bede P, McMackin R, Dukic S, Vajda A, Heverin M, Hardiman O. Cognitive reserve in amyotrophic lateral sclerosis (ALS): a population-based longitudinal study. Journal of Neurology, Neurosurgery & Psychiatry. 2021 May 1;92(5):460-5.

5.1. Introduction

5.1.1. It remains unclear when cognitive impairment emerges and the extent to which cognition declines in ALS. Recent studies suggest that cognitive decline coincides with disease stage (using both Kings and MiTos staging systems) (37,38), although these data are based on cross-sectional design. Longitudinal studies have generally found that cognitive impairment emerges early in the disease. Those who are impaired at diagnosis subsequently decline over time while those who are unimpaired at diagnosis appear to remain stable throughout their illness (39,40,42,264). These studies are often limited by high attrition rates, biasing estimates of true decline due to drop-out of those with faster disease progression.

5.1.2. Joint longitudinal and time to event models (referred to as joint models) have recently gained popularity for their utility in handling data missing-not-at-random and improving statistical power (265). Applying joint modelling to longitudinal neuropsychological data in ALS can give a less biased estimate of the true rate of cognitive decline.

5.1.3. A potential mediator of cognitive change, that may account for some of the varied presentations in ALS, is cognitive reserve (CR). Theories of CR stipulate that neural enrichment, through lifestyle factors such as education, occupation and physical activity, increases an individuals' neural resources (e.g., greater grey matter volume or white matter tract integrity) (45). As the disease spreads across brain regions and networks, individuals recruit these reserve neural resources to deal with cognitive demands, a process known as compensation. Educational attainment, occupational

complexity and leisure activity are often used as proxy measures of cognitive reserve, either in isolation or combined to form a latent construct.

5.1.4. While never studied directly, there is already some indirect evidence that cognitive reserve plays a protective role in ALS. ALS patients with co-morbid FTD, have lower educational attainment and poorer survival (48). Executive functioning and verbal memory are protected by CR in a range of other neurodegenerative conditions such as Parkinson's, Huntington's and Alzheimer's disease (48–52).

5.1.5. This study examined the relationship between CR and longitudinal neuropsychological performance in ALS. Higher cognitive reserve was hypothesized to be associated with improved cognitive performance at baseline, and with a reduced slope of cognitive decline over time. A multiple variable model of CR was applied, using the shared variance of several proxy variables, to create a 'latent' measure of CR. This provides a less biased measure than using an individual proxy in isolation (266).

5.2. Methods

5.2.1. Participants

5.2.1.1. One hundred and eighty-nine ALS patients were recruited for this longitudinal, population-based research study. Participants were recruited as part of ongoing longitudinal studies of neuropsychological deficits in an Irish ALS cohort from 2012-2019 (39). Participants were assessed within the first year of their diagnosis and reassessed 4 times, at least 4 months apart.

Assessments took place in a quiet room in Beaumont Hospital, Dublin, Ireland, or in the participant's home.

5.2.1.2. Exclusion criteria included: 1) history of an intellectual or learning disability, 2) history of a co-morbid neurological, psychiatric or medical condition affecting cognition, 3) alcohol dependence syndrome, 4) if the person was a non-native English speaker or 5) a co-morbid diagnosis of FTD.

5.2.1.3. Occupational history, education and physical activity data were derived from Irish EuroMOTOR project data, a study of environmental risk factors of ALS (267). Clinical data was accessed through the Irish ALS register.

5.2.2. Materials

5.2.2.1. Cognitive reserve score was calculated by combining traditional proxies of CR, namely: education, occupation and physical activity.

Education score was the total years an individual spent in full time education. Occupation was coded according to complexity using the International Standard Classification of Occupations (ISCO) - 88, inverted so that jobs with increasing complexity had higher scores, and then classified into 9 categories ranging from 1 (Elementary Occupations) to 9 (Legislators, senior officials and managers). Occupation score was the sum of years a person spent in an occupation multiplied by its category. This approach matches how the cognitive reserve index questionnaire (CRIq) was developed (268), capturing the time an individual spent in a job as well as the cognitive complexity of that job. Physical activity score was the total

number of years a person reported as engaging in each sport/exercise. Each CR subdomain was converted to a z-score using the sample mean and standard deviation and then averaged to give a CR total score.

5.2.2.2. In the absence of direct biomarkers of the underlying spread of pathologic changes, disease progression was approximated using time from disease onset to each neuropsychological assessment timepoint (266), as described in previous studies (49,269,270).

5.2.2.3. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was used to measure cognition. The ECAS is a sensitive screening tool of cognition in ALS (33). It measures 3 ALS specific functions, language, verbal fluency and executive functioning, and 2 ALS non-specific functions, memory and visuospatial functioning. To examine cognition over time, while limiting the influence of practice effects (as shown in Chapter 4), alternative ECAS versions were used, i.e., ECAS A-B-C (34,271).

5.2.2.4. Participants were also administered a full neuropsychological assessment, consisting of the FAS verbal fluency test (272), the Boston Naming Test (BNT) (273), the Reading the Mind in the Eyes Test (RMET) (274), the Colour-Word Interference Test (CWIT) (275), the Logical Memory test from the Wechsler Memory Scale-III and the Rey Auditory Verbal Learning Test (RAVLT) (276,277). These provided accurate measurements of executive functioning, verbal fluency, language, memory, and social cognition.

5.2.2.5. To limit the influence of motor disability, verbal fluency index (vfi) was calculated on verbal fluency tasks and total errors was used instead of completion time on the CWIT, as described in previous studies (248). Cognitive performance raw scores were converted to z-scores using test manuals and published age, IQ and gender matched healthy control normative data (272–277). A summary of each neuropsychological test, the cognitive domain it measures, and a brief description of the task can be found in table 5.1. below.

Table 5.1. Neuropsychological battery used in cognitive reserve study.

Cognitive Domain	Test Name	Description
Cognitive Screening	ECAS total	Composite score of ALS specific and ALS non-specific scores.
	ECAS ALS specific	Includes short tests of language, verbal fluency and executive functioning.
	ECAS ALS non-specific	Includes short tests of visuospatial functioning and memory.
Verbal Fluency	FAS (Unrestricted) fluency*	Generate as many words as possible beginning with F, A and S, 1 minute for each letter.
	Restricted fluency*	Generate as many words as possible beginning with C, with only 4 letters. Given 1 minute.
Language	Boston naming test (BNT)	Name 30 items.
Social Cognition	Reading the mind in the eyes test (RMET)	Infer the emotional state from 30 faces, given 4 options on each item.
Memory	Logical Memory (LM) WMS-III ^a -Immediate Recall	Recall two short stories, immediate recall and delayed recall after 20 minutes
	Logical Memory (LM) WMS-III - Delayed Recall	
	List recall RAVLT ^b Immediate Recall	Recall a list of 15 words 5 times, delayed recall after 20 minutes
	List recall RAVLT Delayed Recall	

Executive Functioning	Colour-Word Interference Test (CWIT) inhibition**	Must identify the ink colour of incongruent word-colour pairs.
	Colour-Word Interference Test (CWIT) switching/Inhibition**	Must switch between two rules; identify the ink colour or name the word of incongruent word-colour pairs

^a Wechsler Memory Scale, Third version

^b Rey Auditory Verbal Learning Test

* Verbal fluency index (vfi) was calculated to account for motor impairment

** Total errors used instead of completion time to control for bulbar impairment

5.2.3. Statistical analysis

5.2.3.1. Linear mixed effects models were used to examine the association between CR and neuropsychological performance over the course of a year. Linear mixed effects models are a robust means of dealing with missing-at-random data. Additional methods to cater for non-linear associations were applied (see supplementary statistical analysis). In these models the intercept and slope were fitted as random effects, allowing them to vary for each individual. Each mixed model included fixed effect terms for time, age, CR score and an interaction between CR and time. The fixed effect term for CR represents the association between baseline neuropsychological performance and CR. The time by CR interaction term represents the association between the slope of change over time and CR. A Cox survival model was constructed with survival as the outcome variable, and known risk factors for shorter survival as predictors, namely age (278), diagnostic delay (279), site of onset (280), and C9orf72 repeat expansion status (281).

5.2.3.2. The longitudinal mixed models and Cox survival models were then used to create joint longitudinal and time-to-event models (joint models),

using the R package JMBayes (282). This analysis enabled the model to control for non-random dropout in longitudinal data (e.g., individuals who may have dropped out due to a quicker disease progression), resulting in less biased effect estimates. Joint models also provide a measurement of the association between each cognitive measure and survival (283). Analyses were carried out using R statistical software version 3.6.3.(284).

5.3. Results

5.3.1. Demographic Information

5.3.1.1. Participant demographic information at each time point is provided in table 5.2. below. Baseline assessment was carried out ~19 months after their first symptoms (this is due to diagnostic delay). Attrition rate was 79% over the course of the study, indicative of the rapidly progressive nature of the disease.

Table 5.2. Demographic and clinical characteristics, including age, education, sex, site of onset, time from onset to baseline, familial/sporadic family history and C9orf72 status at each time point.

Participant characteristics		Time 1	Time 2	Time 3	Time 4
Age, mean years (SD)		63.59	61.64	60.52	58.94
Education, mean years (SD)		13.8	13.97	14.28	13.94
Sex, n	Male	119	77	54	34
	Female	70	40	27	15
Site of onset, n	Bulbar	44	24	12	4
	Spinal	145	93	69	45
Time from onset to assessment, mean		19.48	25.4	30.11	34.53
ALSFRS-R, mean (SD)		36.5 (7.6)	33.43	32.34	31.4
Delta ALSFRS-R, change in ALSFRS-		-	0.52	0.23	0.21
Familial/Sporadic, n	Familial	31	23	17	13
	Sporadic	158	94	63	36
C9orf72 Status, n	Positive	15	11	6	4
	Negative	174	106	75	45
El Escorial Diagnostic Status, n	Definite	92	50	33	23
	Probable	59	44	29	14
	Possible	38	23	18	12
Strong Criteria Status, n	ALSn	140	89	67	43
	ALSci	33	19	9	5
	ALSbi	6	2	1	0
	ALScbi	10	7	3	1

ALSFRS-R – ALS Functional Rating Scale -Revised; ALSn – ALS with normal cognition and behaviour; ALSci – ALS with cognitive impairment; ALSbi – ALS with behavioural impairment; ALScbi – ALS with cognitive and behavioural impairment

5.3.2. CR as a Predictor of Cognition at Baseline

5.3.2.1. CR score was a significant predictor of baseline performance on the ECAS (including ALS specific and non-specific sub-scores), reading the mind in the eyes test, colour-word interference test (inhibition and switching scores), Boston naming test, and logical memory (immediate and delayed recall). Higher CR was positively associated with higher test performance. CR was not a significant predictor of baseline verbal fluency or RAVLT scores (see table 5.3.).

Table 5.3. Cognitive reserve fixed effect for each neuropsychological test from joint model summary*.

Outcome	β (SE)	95% CI	p
ECAS total	0.37 (.003)	0.11 – 0.64	.003
ECAS ALS specific	0.40 (.002)	0.21 – 0.59	<.001
ECAS ALS non-specific	0.26 (.003)	0.06 – 0.44	.01
Reading the mind in the eyes	0.66 (.005)	0.26 – 1.04	.002
Verbal fluency (unrestricted)	0.49 (.007)	-0.15 – 1.16	.13
Verbal fluency (restricted)	0.61 (.02)	-0.73 – 1.99	.39
CWIT inhibition	0.87 (.008)	0.27 – 1.49	.009
CWIT switching	0.63 (.09)	0.17 – 1.07	.008
Boston naming task	0.55 (.005)	0.13 – 0.98	.01
RAVLT total	0.29 (.004)	-0.02 – 0.59	.07
RAVLT delayed	0.27 (.004)	-0.15 – 0.58	.25
Logical Memory immediate	0.38 (.004)	0.02 – 0.72	.04
Logical Memory delayed	0.56 (.005)	0.11 – 0.99	.03

CI – Credibility Interval

* Full model summaries are provided in supplemental table 6.

5.3.3. CR as a Predictor of Longitudinal Cognition

5.3.3.1. Longitudinal neuropsychological performance, adjusted for age, site of onset, diagnostic delay and C9orf72 status through joint modelling, are displayed in figure 5.1.

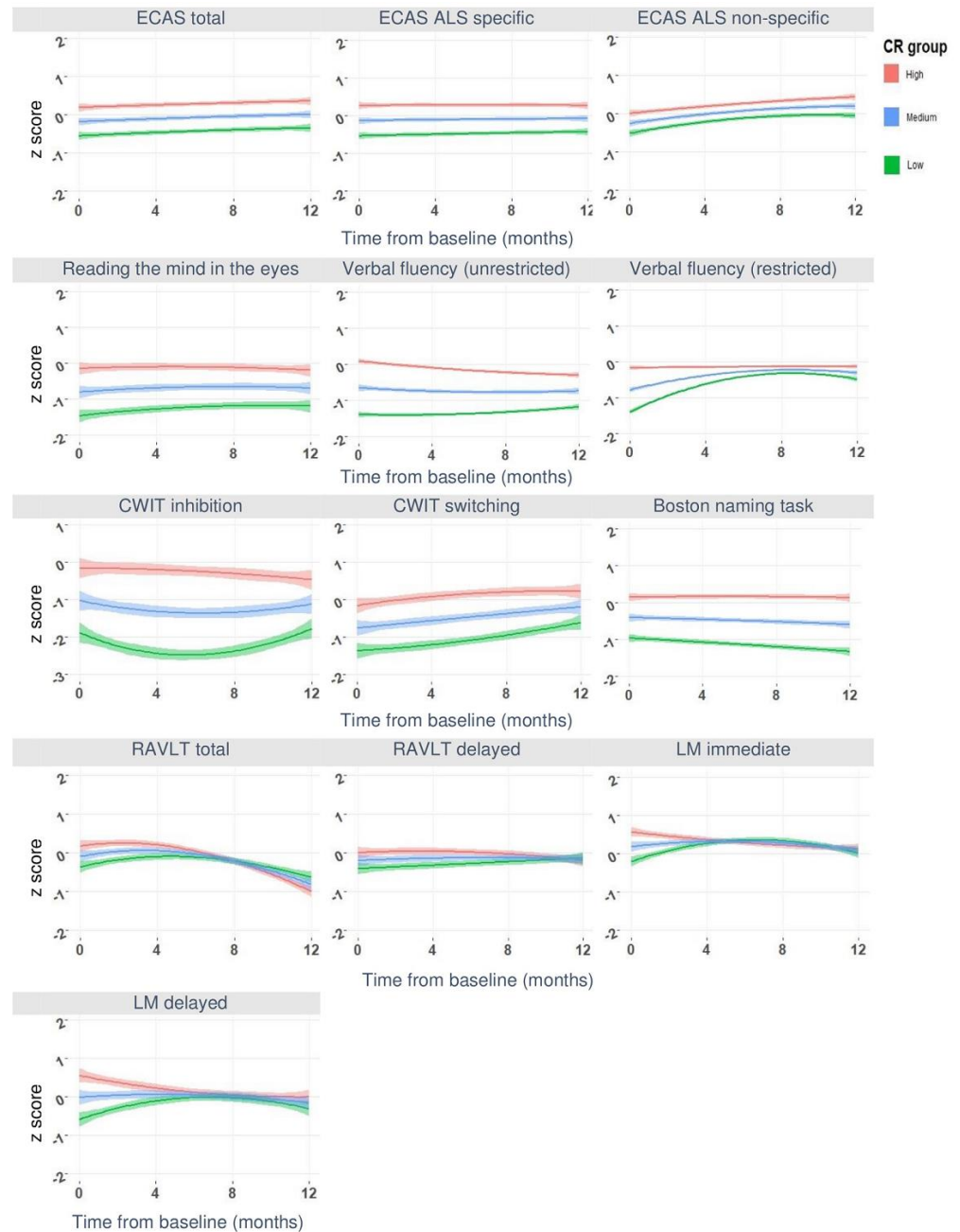


Figure 5.1. Cognitive scores over time in high, medium and low CR groups. High CR defined as having a CR Z score >1 ; medium CR defined as having a CR Z score between $+1$ and -1 ; low CR defined as having a CR Z score <-1 . ALS, amyotrophic lateral sclerosis; CR, cognitive reserve; CWIT, Colour-Word Interference Test;

ECAS, Edinburgh cognitive and behavioural ALS screen; LM, logical memory; RAVLT, Rey Auditory Verbal Learning Test.

9.3.3.2. Individuals were divided into high (CR z-score greater than 1), medium (CR z-score between -1 and 1) and low (CR z-score lower than -1) CR groups to illustrate the effect of CR over time. For ECAS, reading the mind in the eyes test, colour-word interference test and Boston naming test, high CR individuals displayed greater performance than medium and low CR groups over time. For memory tasks, high CR groups declining while low CR groups remained more stable.

5.3.4. Survival risk factors

5.3.4.1. Joint models rely on the construction of Cox survival models to control for non-random drop-out. Controlling for the effects of age, diagnostic delay, site of onset, and C9orf72 repeat expansion status, lower ECAS ALS-specific and RAVLT total score were associated with shorter survival (see table 5.4.). Conversely, higher logical memory immediate score was associated with shorter survival. Table 5.4. displays the association between each cognitive score and survival based on joint modelling (using JMBayes package specified with a current value association structure).

Table 5.4. Estimate of Hazard ratio (HR) for neuropsychological scores from joint models.

Variable	HR (95% CI)
ECAS total	0.88 (0.73 – 1.06)
ECAS ALS Specific	0.65 (0.55 – 0.77) ***
ECAS ALS-Non-Specific	0.99 (0.84 – 1.16)
Reading the Mind in the eyes	1.17 (0.89 – 1.64)
Verbal fluency (unrestricted)	0.93 (0.85 – 1.02)
Verbal fluency (restricted)	1.04 (0.99 – 1.25)
CWIT inhibition	1.09 (0.89 – 1.32)
CWIT switching	0.99 (0.97 – 1.01)
Boston naming test	0.99 (0.86 – 1.17)
RAVLT total	0.99 (0.92 – 0.99) ***
RAVLT delayed	0.81 (0.55 – 1.02)
Logical Memory immediate	1.80 (1.17 – 3.07) ***
Logical Memory delayed	1.39 (0.57 – 2.68)

*** p < .001

5.4. Discussion

5.4.1.1. This study found that cognitive reserve is associated with differences in neuropsychological performance at baseline, and over time, in a population-based cohort. These differences were most notable (i.e., the largest effect sizes were observed) for ECAS, social cognition, executive functioning and confrontational naming tasks. In these domains, high CR individuals had better performance at baseline, and over time. Surprisingly, verbal fluency was not significantly associated with CR, despite the fact that this test is often the most sensitive test of cognitive impairment in ALS. Either CR is not protective for verbal fluency, or more likely, the disease has

progressed to the stage where it has overcome any protective effect for this function.

5.4.1.2. For memory tasks, high CR individuals performed better at baseline but then declined to a greater extent than low CR groups over time (who remained relatively stable). This may suggest that CR plays a differential role whereby it is protective for functions commonly affected by ALS (i.e., executive functioning, language and social cognition), but is less influential on less implicated functions, such as memory.

5.4.1.3. ECAS ALS-specific deficits were associated with shorter survival, consistent with previous studies (59), as was RAVLT total score.

Surprisingly, higher logical memory immediate score was associated with shorter survival. While executive impairment is a known risk factor for shorter survival in ALS, the role of memory is less established. Similar findings have been observed in Alzheimer's research, where individuals with higher CR perform better on memory tasks in the early disease stage, but then suffer from a more rapid disease decline (285).

5.4.1.4. These findings in an ALS cohort, correlate well with those of other neurodegenerative diseases. In Parkinson's disease, high CR patients perform better on attention, executive functioning and visuospatial tasks, and show a slightly reduced rate of decline over time (49). In Huntington's disease, higher CR is associated with better cognition in patients, and in prodromal gene carriers over time (50). Indeed, the effect of CR is most apparent as asymptomatic gene carriers begin to undergo phenoconversion (i.e., manifest symptoms).

5.4.1.5. In Alzheimer's disease there is evidence of a different pattern of change over time. Higher CR is associated with better performance at baseline, but this is followed by a sharper rate of decline once symptoms emerge (285). This suggests that CR may operate by delaying the onset of clinical symptoms rather than reducing the overall rate of decline.

5.4.1.6. The underlying processes that contribute to preserved cognition in high CR individuals remain unclear, but could relate to overall network integrity, mediated in part by preserved grey matter volume (45). In such a case, high CR individuals would be better able to compensate for disease mediated neuronal injury and network disruption.

5.4.1.7. The results of this study support previous longitudinal studies in ALS that those with unimpaired cognition remain relatively stable over time, at least following diagnosis (39,40,264). The utilization of joint models to control for non-random drop-out, suggests that the lack of decline is not solely attributable to the drop-out of highly impaired patients.

5.5. Limitations

5.5.1. Analysis of cognition was limited to 16 months follow-up; and therefore, did not characterize the rate of decline over the longer-term disease course. While unavoidable, the long period between symptom onset and ALS diagnosis, where cognitive impairment is likely to emerge was missed. This study is also limited by the lack of a control group which would have given an indication of practice effects and the extent to which scores normally regress towards the mean. Future studies should compare the role

of CR over a longer period, and relative to healthy controls and similar patient groups.

5.5.2. Disease progression was approximated using time since symptom onset to the point of neuropsychological assessment. This assumes a linear relationship between time and disease progression, which is not often the case. Furthermore, this study is limited by the lack of neuropathological and neuroimaging data. Further work will be required to characterize the relationship between CR and the underlying neuropathological, neuroimaging and neuroelectric changes associated with cognitive impairment in ALS. Studies may consider examining if CR moderates the relationship between DT MRI abnormalities in the corpus callosum and frontotemporal tracts and neuropsychological outcomes (87). Or they could explore if CR mediates the association between ^{18}F -FDG-PET measured hypometabolism in the frontal cortex and cognitive impairment (286–288).

5.6. Conclusions

5.6.1. These findings indicate that higher CR is associated with better neuropsychological performance, particularly in the domains associated with ALSci. Given the limitations of the study, future research is required to explore the relationship between CR and the underlying neuroimaging, neuroelectric and neuropathological signatures of cognitive impairment in ALS.

6. Chapter 6: Endophenotypes in ALS: Study methods

6.1. Introduction

6.1.1. This chapter outlines the methods used to examine endophenotypes in ALS. This includes information on how participants were recruited (including exclusion and inclusion criteria), details on the neuropsychological and neuropsychiatric assessments that were administered, the procedure that was followed (including how genetic testing was carried out), what ethical considerations were relevant and how data were managed and analyzed.

6.2. Study design

6.1. This study utilized a quantitative, observational, cross-sectional, family-based research design. First- and second-degree relatives of ALS patients were compared to healthy controls on a battery of neuropsychological and neuropsychiatric measures.

6.3. Participants

6.3.1. Recruitment

6.3.1.1. ALS patients were identified through the Irish ALS register. With the patient's consent, first- and second-degree relatives were contacted and informed about the research project. Interested individuals were sent a copy of the participant information sheet (see appendix 1) and consent form (see appendix 2). They were re-contacted after 2 weeks to discuss the project and answer any queries. Once the person indicated that they wished to participate, a time and date were arranged to carry out the assessment.

Individuals had the option to complete the assessment in their home, or in a quiet room in Beaumont hospital or Trinity Biomedical Sciences Institute. Most assessments were carried out in the participant's home.

6.3.1.2. Healthy controls were recruited through existing case control projects, and via established recruitment networks, such as local advertising and community groups. Potential participants were contacted by phone or email depending on the contact details they provided. If the person was interested, the control information sheet (see appendix 3) and consent form (see appendix 4) were sent via post or email and a time and date for the assessment were arranged.

6.3.2. Inclusion and exclusion criteria

6.3.2.1. Inclusion criteria for ALS patients were as follows:

1. Diagnosed with ALS between 2010 and 2018;
2. Meeting criteria for Possible, Probable or Definite ALS according to El Escorial criteria.

6.3.2.2. Inclusion criteria for relatives of ALS patients were as follows:

1. A first- or second-degree relative of an ALS patient;
2. Over 18 years of age;
3. A native English speaker.

6.3.2.3. Exclusion criteria for all participants included:

1. A history of neurologic conditions that affect cognition, e.g., stroke, traumatic brain injury, severe epilepsy, etc;
2. A history of a learning disability or developmental disorder;
3. Alcohol dependence syndrome;
4. Severe mental illness;
5. Current use of neuroleptic or psychoactive medication;
6. Under the age of 18.

6.3.2.4. For healthy controls, an additional exclusion criterion was a family history of ALS or FTD.

6.4. Measures

6.4.1. Demographic and clinical information

6.4.1.1. A semi-structured interview was conducted to capture demographic and clinical information. Information was recorded on a demographic and clinical information form. Demographic details included the participant's date of birth, gender, education (years in formal education and highest qualification), occupation and marital status. Clinical details included medication, alcohol intake (measured in units per week); history of depression (and the severity); history of diabetes mellitus, hypertension and hypercholesterolemia; history of head trauma or exposure to heavy metals; and history of intellectual or learning disability.

6.4.2. Neuropsychological assessment

6.4.2.1 The neuropsychological assessment consisted of an extensive battery of cognitive tests (see table 6.1. for a summary of the tests). The assessment began with the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), a sensitive screening tool for cognitive impairment in ALS (33). The assessment then examined 5 key cognitive domains: intellectual functioning, executive functions, language, memory and social cognition. Anxiety and depression were assessed using self-report questionnaires and behavioural change was measured using an informant reported questionnaire. Each cognitive domain and the measures used to assess them are detailed in the following sections.

Table 6.1. Summary of the tests used in the neuropsychological battery and the cognitive function they measure.

<u>Neuropsychological Test</u>		<u>Cognitive Function assessed</u>
Cognitive Screening		
Edinburgh Cognitive and Behavioural ALS Screen (ECAS)		Screening tool of language, executive functioning, verbal fluency, visuospatial ability and memory.
Intellectual Functioning		
Test of Premorbid Functioning UK (TOPF-UK)		Premorbid intellectual functioning
Wechsler Adult Scale of Intelligence 2 nd Edition (WASI-II)	Vocabulary	Semantic knowledge, verbal comprehension and expression.
	Matrix Reasoning	Non-verbal abstract problem solving and inductive reasoning.
Executive Functioning		
Verbal Fluency	FAS Test	Series of lexical and semantic fluency tasks with unrestricted and restricted paradigms.
	Restricted VF (Letter C)	
	Animal fluency	
	Colour Naming	

Colour Word Interference Task (CWIT)	Word Reading	Speed of processing, inhibitory control, error monitoring, selective attention and cognitive flexibility.
	Inhibition	
	Inhibition/Switching	
Digit Span (from the WAIS-IV)	Forward span	Attention and working memory.
	Backward span	
	Sequential span	
Sustained Attention to Response Task (SART)		Sustained attention.
Iowa Gambling Task (IGT)		Emotion based decision making and the somatic marker.
Language		
Boston Naming Task (BNT)		Confrontational naming/word retrieval.
Memory		
Rey Auditory Verbal Learning Test (RAVLT)		Encoding, attention, recall and recognition of verbal information.
Wechsler Memory Scale-3 rd Edition (WMS-III)	Logical Memory I and II	Encoding, attention, recall and recognition of verbal information.
Rey Complex Figure Test (RCFT)		Visuospatial and constructive ability, and visuospatial memory.
Social Cognition		
Reading the Eyes in the Mind Task (RMET)		Ability to perceive, recognize and name facial affect, i.e., theory of mind.
Behaviour		
Beaumont Behavioural Index (BBI)		Behavioural change.
Mood		
Hospital Anxiety and Depression Scale (HADS-T)		Anxiety and Depression.
General Health Questionnaire: 12 item version (GHQ-12)		Screen of non-psychotic and minor psychiatric disorders.

WAIS-IV, Wechsler Adult Intelligence Scale – 4th edition

6.4.2.2. Cognitive screening

6.4.2.2.1. Cognitive screening was carried out using the ECAS, a sensitive screening tool, capable of detecting cognitive impairment in ALS patients (248). The ECAS consists of 5 sub-domains: language, verbal fluency,

executive functioning, memory and visuospatial functioning. Language, verbal fluency and executive functioning are summed to give an ALS-specific score. It is labelled ALS specific as these are the cognitive functions considered to be differentially impaired in ALS patients (33). Memory and visuospatial functioning scores are summed to give an ALS non-specific score. It is labelled ALS-non-specific as these functions are considered relatively preserved in ALS patients. ALS specific and ALS non-specific scores are summed to give an ECAS total score.

6.4.2.3. Intellectual functioning

6.4.2.3.1. Premorbid intelligence was assessed using the Test of Premorbid Functioning-UK version (TOPF-UK) (289), while current intellectual functioning was assessed using the Wechsler Abbreviated Scale of Intelligence 2nd Edition (WASI-II) (290).

TOPF-UK: participants are asked to read a list of 60 words. As the test progresses, the words progressively become more difficult to pronounce. The test is discontinued if the participant gets 5 consecutive pronunciations wrong. Participant's raw score, age, education and gender are entered into the TOPF-UK scorer (a computer application) to calculate a predicted Full-Scale Intelligence Quotient (FSIQ), a Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI) and Processing Speed Index (PSI).

6.4.2.3.2. From the WASI-II, only the Vocabulary and Matrix Reasoning subtests were administered. These two scores were converted to t-scores using age-matched normative data provided in the test manual (290). The

Vocabulary and Matrix Reasoning subtest t-scores were then summed and converted to a Full-Scale Intelligence Quotient - 2 subtest (FSIQ-2) score using the WASI-II user manual.

Vocabulary: participants are asked to define the meaning 31 items (3 pictures and 28 words). Words became more difficult to define as the test progresses. The task examines an individual's word knowledge and verbal concept formation (290). Participants can score 2 points for correctly defining the word, 1 point for partially defining the word, or 0 points for inaccurately defining it. The subtest is discontinued following 3 consecutive scores of 0.

Matrix Reasoning: this subtest has 30 items, each item containing an incomplete matrix. Participants are required to complete the matrix using 1 of 5 picture options provided. Matrix Reasoning provides a measure of fluid intelligence, broad visual intelligence and perceptual organisation (291). The subtest is discontinued following 3 incorrect responses.

6.4.2.4. Executive functioning

6.4.2.4.1. Executive functioning refers to a group of complex mental processes that facilitate goal mediated behaviour. Models of executive functioning typically include processes such as planning, working memory, inhibitory control, flexible thinking and attentional control (292). Many of these processes are mediated by key regions of the fronto-striatal circuits (discussed in Chapter 2), such as the dorsolateral prefrontal cortex (293), the anterior cingulate cortex (294) and the orbitofrontal cortex (295).

6.4.2.4.2. Verbal fluency

6.4.2.4.2.1. Verbal fluency was measured using three paradigms: the FAS test (296), a restricted phonemic fluency task (226) and semantic fluency (297). Verbal fluency is a sensitive marker of cognitive impairment in ALS (226), and is underpinned by frontal and temporal lobes. Frontal lobe functioning is primarily involved in phonemic fluency (the FAS test and restricted fluency), while the temporal lobe is more involved in semantic fluency (298). Verbal fluency scores were converted to z-scores using published age and education derived normative data (297).

FAS test: participants are asked to generate as many words as they can beginning with letters F, A and S. Participants are given 1 minute for each letter and instructed that they cannot use the name of people, places or numbers. If they break one of these rules it is considered a set-loss error, and if they repeat a word, it is deemed a repetition error. Participants are also asked to read the list of words they generated for each letter as quickly as they can. This is used to calculate a person's verbal fluency index (vfi) which accounts for motor impairments. The formula for the vfi calculation is as follows:

$$VFI = \frac{60 - \text{the time taken to read words}}{\text{the number of words}}$$

Restricted fluency task: this is a more difficult version of the FAS test.

Participants are asked to generate as many words as possible in 1 minute that begin with the letter C. However, the words must be only 4 letters long. No

published normative data was available for this paradigm, therefore raw vfi score was used in analysis.

Semantic fluency (Animals): In this version of the fluency paradigm, participants are asked to name as many animals as they can in 1 minute. Semantic fluency score was converted to z-scores using published age and education derived normative data (297).

6.4.2.4.3. Inhibition/switching

6.4.2.4.3.1. Inhibition and switching processes were measured using the Colour Word Interference Test (CWIT) from the Delis-Kaplan Executive Functions System (D-KEFS) battery (275). The CWIT consists of 4 subtests: colour naming, word naming, inhibition and inhibition/switching subtests. There are two types of errors a person can make: self-corrected errors, where the person acknowledges the mistake and corrects it; and uncorrected errors, where they fail to notice the mistake. The time taken to complete each subtest, and the number of errors, were recorded. Raw scores were converted to scaled scores using D-KEFS age-matched normative data provided in the test manual (299).

CWIT colour naming: participants are presented with a sheet of 50 coloured squares. The squares are coloured red, blue or green. The participant is asked to name the ink colours as quickly as they can.

CWIT word naming: participants are presented a sheet of 50 colour words. The words are printed in black ink and spell out 'red', 'blue' or 'green'. The

participant is required to read out loud the list of words as quickly as possible.

CWIT inhibition: participants are presented a list of 50 colour words, where the colour of the ink that the word is presented in does not match what the word is spelled (e.g., the word 'red', printed in a green ink). The participant is instructed to name the ink colour, and not to read what the word spells out. This requires the person inhibit their automatic and dominant response, which is usually to read the word.

CWIT inhibition/switching: this subtest is similar to the inhibition subtest; however, some words are surrounded by a box. Participants are instructed that when the word is not in a box, they must name the ink colour (as was the case in the inhibition subtest). However, when the word is in a box, they must read out loud what the word spells. This requires an individual to inhibit their dominant response in some cases, whilst also being able to switch and follow a new rule.

6.4.2.4.3.2. Performance on the CWIT is mediated by both the dorsolateral prefrontal cortex and the anterior cingulate cortex. The dorsolateral prefrontal cortex is specifically involved in maintaining relevant goal-based information in short term memory and inhibitory control. The anterior cingulate cortex is responsible for response production and attentional allocation (300).

6.4.2.4.4. Working memory

6.4.2.4.4.1. Working memory refers to the ability to hold information in short term memory whilst being able to perform complex tasks with that information (301). To assess working memory, participants were administered the digit span subtest from the Wechsler Adult Intelligence Scale 4th Edition (WAIS-IV) (302). The digit span test has three different conditions: forward, backward and sequential spans. In all conditions, the length of the number series gets longer as the task progresses. The task is discontinued if the participant gets two number series of the same length incorrect.

Digit span forward: a string of numbers is called out. The participant is instructed to call back that string of numbers in the same order that it was read to them.

Digit span backward: a string of numbers is called out. The participant is instructed to call back the string of numbers in reverse order.

Digit span sequential: a string of numbers is called out. The participant is instructed to call back the string of numbers in order of lowest to highest.

6.4.2.4.4.2. A participant's score was measured by the total number items they got correct and the longest number series they got correct (i.e., their longest span). Their longest span was converted to z-scores using age derived normative data provided in the test manuals (302).

6.4.2.4.5. Sustained attention

6.4.2.4.5.1. Sustained attention (or vigilance) refers to a person's ability to engage in effortful attention over a time. Individuals will typically show declining vigilance over time, referred to as the vigilance decrement (303). Sustained attention was assessed using the Sustained Attention to Response Task (SART), a computer-based go/no-go task (304).

SART: participants are presented a series of numbers from 1 to 9 on the centre of a laptop screen. Participants must press the space bar as quickly as they can for every number that appears, except the number 3. When the number 3 appears, they don't press any key. Each number appears for 250 milliseconds with a 900ms interval between numbers. During the interval, a circled X (fixation point) is shown. Participants are shown a practice block of 20 trials, where they are given feedback. After this, the test begins, lasting approximately 4 minutes. The SART was administered using PsychoPy2 software (305), with the test code provided by Cary Stothart via GitHub.com (306).

6.4.2.4.5.2. The SART engages an individual's working memory, sustained attention and inhibitory control (307). The task requires them to actively and consistently attend to their responses so that they can engage inhibitory control over their dominant response. SART performance correlates with right dorsolateral prefrontal cortex and right parietal activity (308). A person's score is measured by their number of commission errors (incorrectly pressing the space bar when 3 is presented), omission errors (failing to press the space bar when any number other than 3 is presented)

and anticipation errors (pressing the space bar too fast). Mean reaction time of the overall test was also recorded.

6.4.2.4.6. Emotion-based decision making

6.4.2.5.6.1. The Iowa Gambling Test (IGT) was administered, via laptop, to examine emotion-based decision making (309). Performance on the IGT can also be viewed from the perspective of the somatic marker hypothesis (310). Somatic markers are feelings in the body that are associated with an emotion (e.g., racing heartbeat when anxious, nausea when disgusted). The somatic marker hypothesis posits that these somatic markers are what often drive decision making, and that this process is mediated by the ventromedial prefrontal cortex and the amygdala (183). From this perspective, poor performance on the IGT may indicate that a person is unable to form or respond to these somatic markers.

IGT: Participants are presented 4 decks of cards on a screen. They must pick one card at a time from one of the 4 decks. They are instructed that for each card they pick, they will win some money; however, on some cards they will win some money and lose some money. They are told that some decks are worse than others and that the goal of the game is to win as much money as possible. Each deck contains a different balance of rewarding and punishing cards. To perform well, participants must learn to favour small reward, small loss decks over large reward, large loss decks. Scores were converted to t-scores using IGT software, derived from age and education matched controls.

6.4.2.5. Language

6.4.2.5.1. Confrontational naming

6.4.2.5.1.1. Confrontational naming was assessed using the 30 item version of the Boston Naming Test (BNT) (273,311). The BNT examines word retrieval and semantic knowledge, and is underpinned by frontal and parietal regions, such as Broca's and Wernicke's areas (298).

BNT (30 item): participants are asked to name 30 increasingly difficult line drawing pictures. If the person is unable to respond spontaneously, they are given a semantic cue (a piece of information about the object). If they are still unable to name the picture, they are then given a phonemic cue (the sound that the word begins with). The participant will have three scores: their total spontaneously correct responses (spontaneous score), their total correct after a semantic cue (semantic score) and their total correct after a phonemic cue (phonemic score). Scores were converted to z-scores using Irish age-matched normative data developed by the Beaumont Hospital psychology department.

6.4.2.6. Memory

6.4.2.6.1. Verbal memory

6.4.2.6.1.1. Verbal memory was assessed using the Rey Auditory Verbal Learning Test (RAVLT) (277) and the logical memory subtest from the Wechsler Memory Scale 3rd edition (WMS-III) (312). The RAVLT task examines an individual's ability to encode, consolidate, store and retrieve verbal information (313).

RAVLT: the participant is instructed to listen carefully to a list of 15 words (list A). When the tester finishes the list, the participant must call back as many words as they can remember (immediate recall). This is repeated 4 more times. Each participant gets a score out of 15 for each trial and a RAVLT total score out of 75 is the sum of these trials. The participant is then given a new distractor list of 15 new words (list B) and asked to call back as many words as they can remember. After this they are asked to call out as many words as they can remember from the original list, i.e., list A. After 20 minutes, participants are asked to call out as many list A words as they can remember (delayed recall). If they remember the full 15, the task is ended. If not, the examiner calls out a list of 50 words containing words from list A and list B, and semantically and phonetically related words. The participant must identify only those words from list A (recognition). Scores were converted to z-scores using published age and gender matched normative data (314).

6.4.2.6.1.2. Performance on distinct components of the RAVLT is dependent on multiple brain networks. Learning in the early trials is associated with cortical thickness in the dorsal attention network; learning in the latter trials is associated with cortical thickness and hippocampus volume; retention is associated with hippocampal volume; and delayed recall is associated with hippocampal volume and cortical thickness of the fronto-parietal network and the default mode network (315).

Logical memory: participants are read a short story and asked to call back as much as they can remember (immediate recall). They are then asked to do

this for a second story. After 20 minutes, the participant is asked to recall as much as they can remember from the two stories (delayed recall). They are then asked 15 questions for each story for which they must answer true or false (recognition). Scores were converted to z-scores using age matched normative data from the test manual (313).

6.4.2.6.1.3. Performance on logical memory relies on episodic verbal memory, tapping memory-encoding, storage and recall processes. The narrative nature of the test is sensitive at detecting cognitive decline in dementia and mild cognitive impairment, as it measures high-order functions such as episodic memory, conceptual organization, and schema formation (316).

6.4.2.6.2. Visuospatial memory

6.4.2.6.2.1. Visuospatial memory was assessed using the Rey Complex Figure Test (RCFT) (317). The RCFT examines an individual's visuospatial ability; spatial and constructive memory and problem-solving strategies (318).

RCFT: participants are asked to copy a complex figure as accurately as they can (copy). The tester records the time taken to complete the drawing. After this, the picture of the figure is put away, and the participant is asked to draw the same figure again as best they can from memory (immediate recall). After 20 minutes, the participant is again asked to draw the figure as best they can from memory (delayed recall). Finally, the participant is shown 24 items, 12 of which are components of the original figure. They are asked to identify which items were from the original figure (recognition). When

scoring, the figure is broken down into 18 scorable components. A participant can score 2 if a component is accurately drawn and accurately placed; 1 point if a component is inaccurately drawn and accurately placed OR accurately drawn and inaccurately placed; ½ a point if the component is inaccurately drawn and inaccurately placed but recognizable; and 0 points if the component is missing or unrecognizable. Scores were converted to z-scores using age matched normative data from the test manual (319).

6.4.2.7. Social cognition

6.4.2.8.1. Social cognition refers to how individuals process, store and apply information about other people and social behaviours. One of the key components of social cognition is theory of mind, which refers to the ability to infer the mental and emotional states of others. In this study, theory of mind was assessed using the reading the mind in the eyes test (RMET) (274). Performance on the RMET is positively associated with grey matter volume in fronto-temporo-parietal networks, specifically the dorsomedial prefrontal cortex, the inferior parietal lobule and the left precuneus (320).

RMET: participants are presented 30 black and white pictures of people's faces, showing from the forehead to the mid-nose of the face. Participants are asked to identify the emotion of the person, picking from 4 options given to them for each item. An individual's score is the total number of items they get correct. Raw scores were converted to z-scores using published Irish control data (321).

6.4.2.8. Behaviour change

6.4.2.8.1. Behavioural change was measured using the Beaumont Behavioural Inventory (BBI) (322) and the Frontal Systems Behaviour Scale (FrSBe) (323). The BBI is an informant report questionnaire, designed to assess the behavioural changes that are most common in ALS, including: apathy, disinhibition, impulsivity, stereotyped behaviour, dietary changes and perceptual disturbances. The FrsBe provides a self-report and informant report of behavioural change, examining apathy, disinhibition and dysexecutive behaviour.

BBI: an informant of the participant, usually a close family member is asked to complete a 41-item questionnaire. The BBI has two columns, asking the informant if the participant has changed their behaviour a) in the last 10 years, and b) since the onset of MND. For relatives of ALS patients and controls, they were given the control BBI version. This asks if the participant has changed a) in the last 10 years and b) in the last 3-5 years. The participant has 4 options for each question, with each getting a different score: No/No change (0), mild (1), moderate (2) and severe (3). All items are summed to give a BBI total score. Published cut-offs provide thresholds for clinically relevant behavioural change (322). Scores below 7 are considered normal, from 7-22 indicates mild behavioural change, and greater than 22 indicates severe behavioural change.

FrsBe: this questionnaire provides a self-rating form and an informant rated form. Participants are asked to rate the extent to which they agree or disagree with 46 statements, comparing before and after their MND diagnosis.

Relatives and controls were given a control version, rating the statements for 3-5 years ago compared to the present time. They respond to these questions on a 5-point Likert scale from 1 (almost never) to 5 (almost always).

6.4.2.9. Mood

6.4.2.9.1. Mood was screened using the Hospital Anxiety and Depression Scale (HADS-T) (324) and the 12 item General Health Questionnaire (GHQ-12) (325,326).

HADS-T: participants are asked 14 questions, 7 relating to anxiety symptoms and 7 relating to depressive symptoms. Participants rate their response in terms of which response best applies to how they have been feeling over the last 2 weeks. These responses are coded from 0 to 3, with higher scores indicating more anxious and depressive symptoms. This version of the HADS is adapted for an ALS patient population. Scores greater between 17 and 20 indicate a possible mood disorder and scores above 20 indicate a probable mood disorder.

GHQ-12: participants are asked 12 questions, designed to identify common psychiatric conditions. Participants respond on a 4-point Likert scale with higher scores indicating poorer mental health.

6.4.3. Neuropsychiatric assessment

6.4.3.1. To examine neuropsychiatric traits, participants were administered an in-depth neuropsychiatric questionnaire. This assessment consisted of questionnaires from the UK Biobank Thoughts and Feelings Questionnaire (327), and seven additional questionnaires (see table 4.2. for summary). The

neuropsychiatric questionnaire was primarily administered online, using the survey platform Qualtrics (328). A paper version alternative was provided if the person was unable to use a computer or declined the online version.

Table 6.2. Neuropsychiatric questionnaires administered and the traits they assessed.

Neuropsychiatric Questionnaire		Psychiatric trait/behaviour assessed
UK Biobank Thoughts and Feelings Questionnaire	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)		Presence of impulsive behaviours and preferences in five domains: attention, cognitive stability, perseverance, self-control and 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 aspects of behavioural and cognitive difficulties associated with autism.
Adult ADHD Self-Report Scale (ASRS)		Symptoms of ADD/ADHD based on the DSM-IV criteria.

Community Assessment of Psychic Experiences (CAPE-P15)	Positive symptoms of psychosis: persecutory ideation, bizarre experiences and perceptual abnormalities.
The Ten Item Personality Inventory (TIPI)	Five main personality traits; Extroversion, openness, agreeableness, neuroticism and conscientiousness.

6.4.3.2. UK Biobank thoughts and feelings questionnaire

6.4.3.2.1. The UK Biobank Thoughts and Feelings Questionnaire is designed to identify clinical and subclinical psychiatric disorders, such as anxiety, depression, psychosis, and OCD. Depression is assessed using the Patients Health Questionnaire-9 (PHQ-9) (329). Anxiety was assessed using the Generalized Anxiety Disorder – 7 (GAD) (330). A history of depression, anxiety, panic attacks and OCD were examined using adapted questions from the Composite International Diagnostic Interview Short Form (CIDI-SF) (331), which is based on Diagnostic and Statistical Manual of Mental Health Disorders fourth edition (DSM-IV) criteria. Psychosis and schizophrenia positive symptoms were assessed using the Psychosis Screening Questionnaire (PSQ) (332). Alcohol use disorder was measured using the World Health Organisation’s (WHO) Alcohol Use Disorders Identification Test (AUDIT) (333).

Patients Health Questionnaire-9 (PHQ-9): Assesses each of the DSM-IV criteria for depression, with item responses ranging from 0 (not at all) to 3 (every day). PHQ-9 total scores from 0-4 indicate no depressive symptoms, 5-9 indicate mild symptoms, 10-14 indicate moderate symptoms, 15-19

indicate moderately severe symptoms and scores 20 or higher indicates severe symptoms. A PHQ-9 score of 10 or higher has 88% sensitivity and specificity for major depressive disorder (329).

Generalised Anxiety Disorder - 7 (GAD): Assesses symptoms of GAD, with item responses ranging from 0 (not at all) to 3 (nearly every day). GAD-7 total scores below 5 indicate no GAD symptoms, 5-9 indicate mild symptoms, 10-14 indicate moderate symptoms, and scores above 15 indicate severe symptoms. A GAD-7 score of 10 or higher has 89% sensitivity and 82% specificity for GAD (334).

6.4.3.3. Additional questionnaires

6.4.3.3.1. An additional 7 questionnaires were included, providing measures of OCD, impulsivity, apathy, autism spectrum, ADHD, psychotic and personality traits.

Obsessive-Compulsive Inventory Revised (OCI-R) (335): participants are asked 18 items, examining numerous obsessions and compulsions, such as washing, checking, ordering, obsessing, hoarding, and neutralising. Participants respond using a 5-point Likert scale (0-4), with higher scores indicating a greater degree of distress from symptoms. Scores are summed to give an OCI-R total score (0-72), with scores above 21 indicating the likely presence of OCD, with 66% sensitivity (335)

Barrett Impulsiveness Scale (BIS-11) (336): participants are given 30 questions examining the presence of impulsive behaviours and preferences. Participants respond on a 4-point Likert scale from 1-4, with a total score

ranging from 30-120 and scores above 72 indicating high impulsivity and a score below 52 indicates a person is extremely over-controlled (337). The BIS-11 measures the multiple dimensions of impulsiveness, with sub-scores for first order factors: attention, motor and non-planning; and second order factors: attention, motor, self-control, cognitive complexity, perseverance and cognitive instability.

Dimensional Apathy Scale (DAS) (338): participants are given 24 items, assessing three dimensions of apathy: executive, emotional, and cognitive/behavioural initiation. Participants respond on a 4-point Likert scale with higher scores indicating greater apathy. Clinically relevant cut-offs are provided for DAS total score, with scores above 39 indicative of clinically meaningful apathy (54).

Autism Spectrum Quotient (AQ) (339): the AQ consists of 50 items, assessing behavioural and cognitive difficulties associated with autism. It examines 5 domains, including attention switching, communication, social skills, attention to detail and imagination. Participants respond on a 4-point Likert scale from definitely agree to definitely disagree. Response's indicative of ASD traits get a score of 1. Participant responses are summed to give an AQ total score ranging from 0-50, with a cut-off score of 32 indicating a possible diagnosis of ASD (339).

Adult ADHD Self-Report Scale (ASRS) (340): participants are asked 6 questions that examine DSM-IV criteria for ADD/ADHD. Participants rate the frequency that each symptom has occurred in the last 6 months on a 5-point Likert scale, ranging from never (0) to very often (4). Items are

weighted to determine a probable diagnosis of ADHD. If a person endorses 4 items, the person has symptoms highly consistent with ADHD. A continuous score from 0 to 24 quantifies symptom severity.

Community Assessment of Psychic Experiences (CAPE-P15) (341):

participants are asked 15 questions about positive symptoms of psychosis.

The CAPE-P15 examines 3 types of symptoms: persecutory ideation, bizarre experiences and perceptual abnormalities. Participants rate the severity of, and distress caused from, psychosis symptoms on a 4-point Likert scale, providing a total score range from 0 to 45.

Ten Item Personality Inventory (TIPI) (342): measures the 5 main

personality traits: extroversion, openness, agreeableness, neuroticism and conscientiousness. Participants rate the extent to which they identify with each trait on a 7-point Likert scale, from disagree strongly (1) to agree strongly (7).

6.5. Procedure

6.5.1. Once participants expressed an interest in the study, a date, time and location were arranged. Participants had the option to carry out the assessment in Trinity Biomedical Sciences Institute, Beaumont Hospital or their own home. In all settings, it was ensured that testing took place in a quiet room where there would be no interruptions or distractions.

6.5.2. At the beginning of the research interview, the tester read through the participant information leaflet, providing ample time for the participant to ask questions. Once the participant was satisfied with the information and

expressed that they wished to take part, they were given the participant consent form. Participants were given a copy of the information leaflet and consent form for their own records.

6.5.3. Once consent was obtained, the tester began the neuropsychological assessment. The measures used in this assessment are detailed in section 6.4.2. The battery of tests took ~2-3 hours to complete. A short 10-minute break was provided half-way through the assessment to minimize fatigue, and additional breaks were offered if the participant became fatigued at any stage.

6.5.5. After the neuropsychological assessment, a sample of blood was taken from participants for genetic testing (see section 6.6.). Participants were given the BBI and FrsBe and instructed that these forms needed to be completed by a family member. Participants were given a stamped envelope and instructed to post the questionnaires back once they were completed.

6.5.6. Lastly, the email address of each participant was obtained. Participants were instructed that they would receive an email with a link to an online ‘thoughts and feelings’ questionnaire (i.e., the neuropsychiatric assessment outlined in section 6.4.3.). On the email, the participant was given an ID code to enter on the first page of the online questionnaire. For participants who did not wish to carry out the questionnaire online, a paper version was provided which participants sent back to testers in the post. The online questionnaire took approximately 30-45 minutes to complete.

6.6. Genetic testing

6.6.1. A blood sample was taken from all participants for DNA extraction and analysis. All samples were given a unique code at source and stored in a freezer in Trinity Biomedical Sciences Institute. DNA samples were tested for the presence of the pathogenic C9orf72 repeat expansion. Participants with greater than 30 hexanucleotide repeats were deemed positive for C9orf72. Expansions with 20 – 29 repeats were considered intermediate C9orf72 carriers. Participants with 19 or less hexanucleotide repeats were deemed C9orf72 negative.

6.6.2. Participants and researchers were not informed of the genetic test results of any individual and the geneticists performing the genetic tests did not have access to participant identifiers. Once data were anonymized for data analysis, genetic status was added to the database by the ALS research manager Mark Heverin. This ensured that all parties were blinded to each individual's genetic status throughout the study.

6.7. Ethical considerations

6.7.1. Ethical approval was granted by Beaumont Hospital Research Ethics Committee (REC Reference 15/40). Informed written consent was obtained from all participants. The participant information leaflet and consent forms were written in plain English to ensure that individuals were fully aware of the rationale, procedures, risks and benefits of the study. Individuals were given a cooling off period of a week to consider the information before consenting. This ensured that they did not feel pressured to sign up to the study straight away.

6.7.2. A data protection impact assessment was completed and approved by the data protection officer in Beaumont Hospital. Participant data was pseudonymized at source, with the key being retained by the research manager. Pen and paper data were stored under lock and key in the Academic Unit of Neurology in the Trinity Biomedical Sciences Institute. Electronic data were encrypted on password protected computers/laptops on secure Trinity College Dublin servers. Participants were made aware they could withdraw from the study at any time, for any reason, and that this would not affect the patient's quality of care. They were also informed that they could withdraw their data at any time.

6.7.3. The research study did not entail any immediate benefit to participants, nor was there any expected harm. Participants may have experienced discomfort and/or bruising when getting their blood sample taken. Participants may also have felt fatigued when completing the neuropsychological assessment. Short breaks were encouraged to mitigate this risk.

6.8. Data scoring and management

6.8.1. Neuropsychological performance was scored according to test manual administration guidelines. Raw scores were converted to z-scores, t-scores, scaled scores or standard scores, depending on what conversion was provided by the test manuals. Raw and converted scores were entered into password encrypted Microsoft Excel files to electronically store and manage the data.

6.8.2. Neuropsychiatric traits collected via the Qualtrics ‘thoughts and feelings’ questionnaire were automatically saved to the Qualtrics account once completed. When data collection was complete, all ‘thoughts and feelings’ data were exported as a Microsoft Excel file and password encrypted. Scores below published cut-offs were labelled as ‘abnormal’.

6.8.3. Clinical data were derived from the ALS register, an ongoing database of Irish ALS patient clinical data (343). Patients consented to the ALS register during their routine visit to the multi-disciplinary ALS clinic in Beaumont Hospital. To be added to the ALS register, patients consented to a structured interview where information on their residency, clinical features, medical care, medical procedures, functional status and family history are collected for the express purpose of research.

6.9. Statistical Analyses

6.9.1. All analyses were carried out using R statistical software, version 3.6.3 (344). The R code used to carry out analyses is provided in Appendix 5 (as the code for each outcome is too long to fully include, code is provided for ECAS and PHQ-9 scores as outcome variables. Analysis of other neuropsychological and neuropsychiatric scores used similar code, with the relevant outcome specified). The following R packages were used: tidyverse, ggplot2, readxl, here, summarytools, rstatix, ggpubr, effectsize, pastecs, car, scales, ggforce, ggpubr, knitr, kableExtra, table1, MatchIt, QuantPsyc, factoextra, NbClust, GGally, plotly.

6.9.2. Sample size considerations and calculation

6.9.2.1. Small sample sizes typically result in large variances, contribute to more type 2 errors (i.e., false negatives) and lead to inaccurate effect estimates. To calculate the minimum sample size needed, one must estimate the expected effect size. However, effect sizes from the literature are often inflated due to publication bias. Consequently, unbiased estimates of effect sizes, such as hedges g , epsilon, or omega, should be used in sample size calculations.

6.9.2.2. Whilst type 1 error is often the primary concern during analysis, type 2 error must also be considered. Unlike type 1 error, which can be controlled for by replications, type 2 errors will result in no future replication studies, meaning true effects can be lost. Increasing sample size is the most common way to improve power (thus lowering type 2 error) but there are other methods, such as reducing measurement error, increasing the variation of responses, using one sided tests and using within subject designs.

6.9.2.3. As discussed in chapter 2, there are no direct studies of cognitive endophenotypes in ALS. Thus, expected effect sizes were estimated from studies of asymptomatic gene carriers and reported effect sizes of endophenotypes in studies of related disorders, such as schizophrenia, suicidal behaviour and OCD. Table 6.3. summarizes 4 key studies that informed the estimated effect size for neuropsychological performance. As there was a wide range of tests implemented in these studies, tests were selected that are sensitive to cognitive impairment in ALS, such as verbal fluency and letter-number sequencing, to inform sample size calculation.

Table 6.4. summarizes 4 key studies that have informed the estimated effect size for neuropsychiatric traits in this study.

Table 6.3. Effect size estimates of potential neuropsychological endophenotypes.

Authors (year)	n	Population	Test	Effect Size	
				Group comparisons	Hedges g (95% CI)
Lulé et al. (2020) (151)	127	ALS gene carriers ^a	Verbal Fluency	C9orf72 carriers vs controls	0.759 (0.21 – 1.31)
				SOD1 carriers vs controls	0.47 (-0.14 – 1.08)
				Non-gene carriers vs controls	0.51 (0.08 – 0.94)
Lee et al. (2017) (345)	61	ALS gene carriers ^b	Verbal Fluency	C9orf72 carriers vs controls	0.11 (-0.47 – 0.70)
Tikka et al. (2020) (346)	200	Schizophrenia ^c	Letter-number sequencing	CAR vs controls	0.97 (0.55 – 1.38)
				FAR vs controls	0.75 (0.35 – 1.16)
Liang et al. (2016) (347)	985	Schizophrenia ^d	Verbal Fluency	FS parents vs controls	1.12 (0.94 – 1.45)

				SS parents vs controls	0.94 (0.73 – 1.15)

^a Included asymptomatic C9orf72 and SOD1 gene carriers, non-gene carrier relatives and healthy controls

^b Included asymptomatic C9orf72 gene carriers and healthy controls

^c Included people Familial-At-Risk (FAR) and Clinically-At-Risk (CAR) of schizophrenia and healthy controls

^d Included familial and sporadic schizophrenia patients and parents, and healthy controls

Table 6.4. Effect size estimates of potential neuropsychiatric trait endophenotypes.

Authors (year)	n	Population	Test	Effect Size	
				Group comparisons	Hedges g (95% CI)
Grossman et al. (2006) (348)	46	ALS ^a	NEO-PI Openness trait	ALS vs lung cancer caregivers	0.23 (-0.28 – 0.73)
				ALS vs MS caregivers	0.69 (0.09 – 1.28)
				ALS vs brain glioma caregivers	0.77 (0.07 – 1.46)
Melhem et al. (2007) (179)	365	Suicidal Behaviour ^b	Buss-Durkee Hostility Inventory Impulsive Aggression	Suicide attempters vs non-suicide attempters	-1.59 (-2.05 – - 1.24)

Rector et al. (2009) (200)	135	OCD ^c	Obsessive beliefs Questionnaire – Perfectionism trait	OCD relatives vs controls	0.34 (-0.1 – - 0.81)
Wheelright et al. (2010) (221)	3007	Autism ^d	Autism Quotient -	ASD mothers vs control mothers	-.33 (-0.46 – -0- 19)
				ASD fathers vs control fathers	-.46 (-0.55 – - 0.37)

^a Included caregivers of ALS, MS, lung cancer and brain glioma patients. Asked to rate premorbid personality of patient

^b Included offspring of individuals with history of mood disorder and suicidal behaviour

^c Included OCD patients, first degree relatives and healthy controls

^d Included parents of children with ASD and parents on typically developing children

6.9.2.4. For this study, an alpha value of 0.05 was adopted. This means that if the null hypothesis is true (i.e., no effect exists), a type 1 error (i.e., a false positive) will be expected 5% of the time. A desired power of 0.8 was specified. If achieved, this means that the study will capture a true effect 80% of the time. This desired power also means an expected type 2 error (i.e., a false negative) 20% percent of the time. Based on the literature highlighted in table 6.3., medium to large effect sizes for neuropsychological performance (average effect size is 0.7) were expected. The literature highlighted in table 6.4. suggests slightly smaller and more variable expected effect sizes for neuropsychiatric traits (average effect size is 0.63).

6.9.2.5. These figures (i.e., alpha level, desired power and estimated effect sizes) were input into G*Power software (Version 3.1.) (349) to calculate the minimum sample size required for this study. Tests were decided to be two-

tailed, as although ALS relatives were expected to perform worse than controls, this was not guaranteed. There was also a possibility that certain ALS sub-groups could perform unexpectedly. In order to carry out a one-way ANOVA, with an estimated effect size of 0.7 (as expected for neuropsychological endophenotypes), an alpha of 0.05 and a desired power of 0.8, a minimum sample size of 139 is required. When the estimated effect size was 0.63 (as expected for neuropsychiatric endophenotypes), the minimum sample needed was 170. To investigate interaction effects or to carry out an ANCOVA to control for covariates, a sample size of 142 was required when the expected effect size is 0.7, and 173 when it is 0.63.

6.9.3. Planned group comparisons

6.9.3.1. The primary analysis compared neuropsychological and neuropsychiatric scores in 2 groups: relatives of ALS patients and healthy controls. Further comparisons were carried out on various subgroups: Familial ALS relatives vs Sporadic ALS relatives, C9orf72 positive vs C9orf72 negative relatives. Participants were compared on numerous measures, thus inflating the probability of making a type 1 error. The Bonferroni correction controls for this issue by dividing the alpha by the number of tests. The Bonferroni-Holm correction is a slightly more accurate method of controlling for multiple comparisons error rate, while maintaining high power. As a result, p-values were adjusted using the Holm-Bonferroni method (350).

ALS relatives vs controls: The first analysis compared relatives of ALS patients and controls using Welch's independent samples t-tests (if data were parametric) or Wilcoxon rank tests (if data were non-parametric).

FALS relatives vs SALS relatives vs controls: This analysis compared relatives of familial ALS patients (as defined by Byrne criteria (351)) with relatives of sporadic patients and controls using one-way ANOVAs (if data were parametric) or Kruskal-Wallis tests (if data were non-parametric). If a significant main effect was observed, post-hoc Tukey's tests were carried out to compare subgroups.

ALS relatives with C9orf72 positive vs negative genetic status: This analysis compared C9orf72 positive relatives and C9orf72 negative relatives using Welch's independent samples t-tests (if data were parametric) or Wilcoxon rank tests (if data were non-parametric). As C9orf72 is a relatively rare mutation, it was possible that the necessary number of C9orf72 positive participants to achieve adequate power would not be met.

6.9.4. Effect size

6.9.4.1. While p-values are essential in determining if an effect is distinguishable from chance variation or not, it does not inform us as to the magnitude of an effect. Effect size is a measurement of the strength of a phenomenon and is typically conveyed by statistics such as Cohens d (to measure mean differences) and Pearson's r co-efficient (to measure the strength of an association). Cohen provides rules of thumb for interpreting effect sizes (352). A Cohens d of 0.2 is typically considered a small effect size, 0.5 a medium effect size, and 0.8 a large effect size. Similarly, a

Pearson's r of 0.1 is typically considered small, 0.3 is a medium effect size, and 0.5 is a large effect size.

6.9.5. Confidence Intervals

6.9.5.1. In addition to wanting to know if an effect is significant, and its magnitude, parameters must be estimated accurately. Confidence intervals compliment p-values as they provide an estimate of the precision of the effect. In other words, they provide a measure of the uncertainty of a statistic. As with p-values, confidence intervals are a frequentalist concept. A 95% confidence interval estimates that, in the long run, 95% of confidence intervals will contain the true population parameter. If 100 replications of this study were subsequently carried out, in 95 of those studies, the true parameter would lie within their confidence intervals; and in 5 studies the true parameter would lie outside their confidence intervals. As sample size increases, confidence intervals will become narrower, thus resulting in a more precise estimate of a parameter. This can be seen in tables 6.3. and 6.4., where the confidence intervals of studies with larger sample sizes are much narrower, and therefore more precise, than studies with smaller samples sizes.

7. Chapter 7. Results 1: Neuropsychological Endophenotypes in Unaffected Relatives of ALS patients

7.1. Introduction

7.1.1. This chapter examined aim 3 of this thesis, which was to identify candidate neuropsychological endophenotypes of ALS. To do this, first and second-degree relatives of ALS patients were compared with controls across a full neuropsychological battery of tests. As this is the first study of its kind in ALS, an exploratory approach was undertaken for data analysis.

7.2. Methods

7.2.1. Participant recruitment, inclusion and exclusion criteria, the neuropsychological assessment that was carried out, and the procedure that was followed are all detailed in chapter 6. ALS relatives and controls were compared across all cognitive domains assessed. To control for an inflated type 1 error rate due to multiple comparisons, Bonferroni-Holm corrections were applied.

7.2.2. Participants

7.2.2.1. In total, 358 participants were recruited and consented to participate. This consisted of 224 first- and second-degree relatives of ALS patients and 134 controls. All consenting participants gave a blood sample for genetic testing and completed at least a cognitive screening assessment. From this sample of 358 participants, 221 completed a full neuropsychological assessment, of which, 161 were relatives of ALS patients and 60 were healthy controls.

7.2.2.2. Ten participants were excluded as they were under 18 years of age.

Three participants were excluded as they reported having dyslexia. ALS family member recruitment, degree of participation and exclusion are summarized in figure 7.1. below.

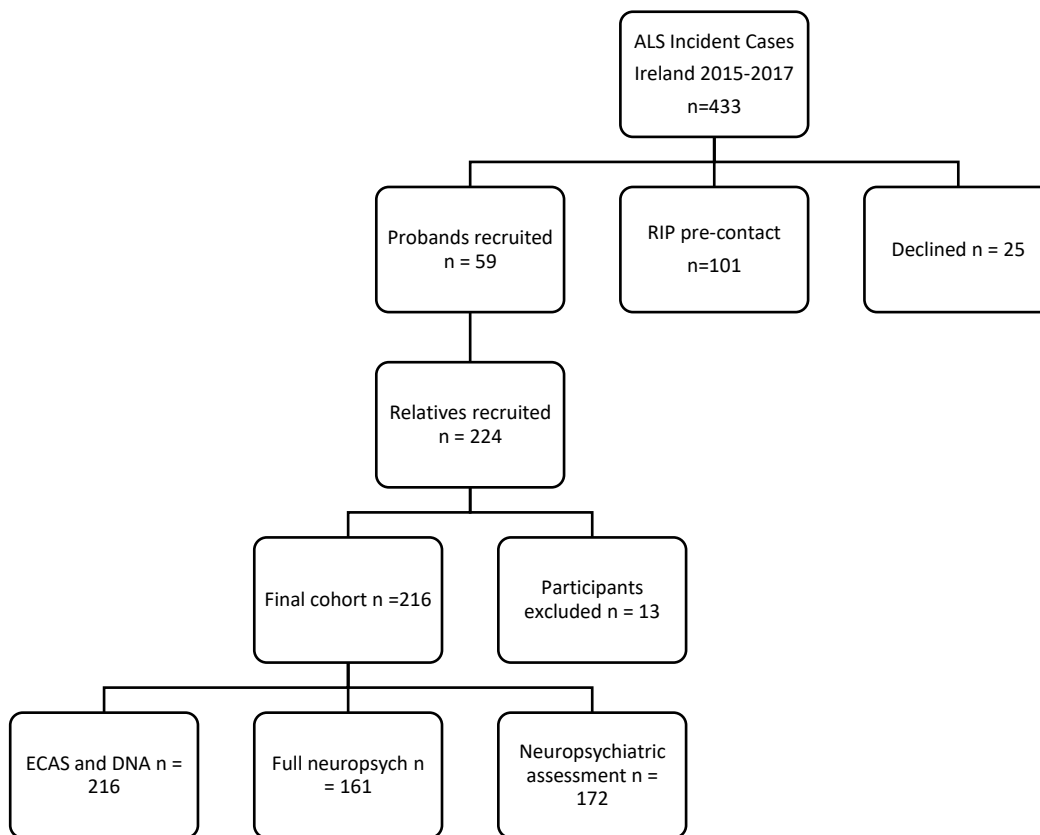


Figure 7.1. Flowchart of participant recruitment and degree of participation.

7.2.3. Statistical analysis

7.2.3.1. Three group comparisons were carried out for each cognitive test.

Firstly, ALS relatives were compared to healthy controls. Secondly, the effect of family history was examined by comparing relatives of Familial ALS patients, relatives of Sporadic ALS patients and healthy controls.

Lastly, the effect of the C9orf72 repeat expansion was assessed by

comparing ALS relatives who were C9orf72 positive and relatives who were C9orf72 negative. For this analysis, individuals with an intermediate C9orf72 status (i.e., between 20 – 29 repeats) were considered as positive. To control for confounding factors such as age, education and gender, neuropsychological performance was converted to z-scores using normative data provided in test manuals and published literature.

7.2.3.2. Between group comparisons were carried out using Welch's t-tests (for 2 groups) and one-way ANOVAs (for 3 groups) where data were parametric and Wilcoxon rank tests (for 2 groups) and Kruskal-Wallis tests (for 3 groups) where data were non-parametric. Welch's t-test is preferable to Student's t-test when the samples sizes in each group are unequal, as is the case here, and it does not rely on the assumption of equal variances. The Bonferroni-Holm method was used to control for inflated type 1 error due to multiple comparisons. Significant differences between groups are illustrated using box and violin plots, and effect sizes are reported as Cohen's d, Pearson's r or omega squared.

7.3. Results

7.3.1. Full sample demographics

7.3.1.1. Demographic and clinical information for the total sample (i.e., participants who gave at least a blood sample and completed an ECAS) is presented in table 7.1. below. The ALS relatives group were majority female and had a lower mean age than the control sample. This is because this cohort consisted of two main subgroups: the siblings of patients and offspring of patients. The cohort of ALS patient offspring meant that the

mean age of the relative group is quite young. In contrast, healthy controls were recruited from an existing control database. This group is largely made up of older, retired individuals. The relatives group had more years of education and a higher frequency of tertiary education.

Table 7.1. Demographic and clinical characteristics of participants with at least an ECAS and DNA sample.

	Controls (n = 134)	Relatives (n = 216)
Gender		
Female, n (%)	53 (39.6%)	119 (55.1%)
Male, n (%)	81 (60.4%)	97 (44.9%)
Age		
Age in years, mean (sd)	61.7 (9.11)	45.1 (16.7)
Handedness		
Ambidextrous, n (%)	1 (0.7%)	2 (0.9%)
Left, n (%)	11 (8.2%)	20 (9.3%)
Right, n (%)	122 (91.1%)	194 (89.8%)
Education		
Years in education, mean (sd)	14.6 (5.07)	16.3 (3.59)
Highest education level		
Primary, n (%)	21 (15.7%)	12 (5.5%)
Secondary, n (%)	61 (45.5%)	59 (27.3%)
Tertiary, n (%)	52 (38.8%)	133 (61.6%)
Apprenticeship, n (%)	0 (0%)	12 (5.6%)
C9orf72Status		
Intermediate, n (%)		9 (4.1%)
Negative, n (%)		163 (75.5%)
Positive, n (%)		31 (14.4%)
Missing, n (%)		13 (6.0%)
Family History		
FALS, n (%)		149 (69.0%)
SALS, n (%)		67 (31.0%)

FALS, Familial ALS; SALS, Sporadic ALS

7.3.1.2. Within the ALS relatives group, 149 were related to an ALS patient with familial ALS, according to the Byrne criteria (351), and 67 were related

to an ALS patient with sporadic ALS. Thirty-one unaffected relatives of ALS patients tested positive for the C9orf72 repeat expansion, 163 tested negative, and 9 had an intermediate C9orf72 repeat expansion (i.e., they had between 20 and 30 repeat expansions).

7.3.2. Demographics of cohort with full neuropsychological assessment

7.3.2.1. Due to the length of the full neuropsychological assessment, many participants (n = 67, 31%) opted out of this part of the study. The participants that opted out were less educated than those who completed the full study and a significant proportion of them were C9orf72 carriers (n = 21). Demographic and clinical information of the participants who completed a full neuropsychological assessment are presented in table 7.2. below. ALS relatives and controls are well matched in respect of gender, handedness, education, marital status and alcohol intake. However, as with the larger cohort, the control group was older than the relatives cohort, with more controls in retirement.

Table 7.2. Demographic and clinical characteristics of participants who completed the full neuropsychological assessment.

	Controls (n = 60)	Relatives (n = 149)
Sex		
Female, n (%)	32 (53.3%)	82 (55.0%)
Male, n (%)	28 (46.7%)	67 (45.0%)
Age		
Age in years, mean (sd)	63.7 (10.2)	46.1 (17.4)
Handedness		
Left, n (%)	8 (13.3%)	18 (12.1%)
Right, n (%)	52 (86.7%)	129 (86.6%)
Ambidextrous, n (%)	0 (0%)	2 (1.3%)

	Controls (n = 60)	Relatives (n = 149)
Education		
Years in education, mean (sd)	16.4 (3.70)	16.7 (3.19)
Highest education level		
Apprenticeship, n (%)	1 (1.7%)	7 (4.7%)
Primary, n (%)	1 (1.7%)	3 (2.0%)
Secondary, n (%)	19 (31.7%)	36 (24.2%)
Tertiary, n (%)	39 (65.0%)	103 (69.1%)
Currently working		
No, n (%)	37 (61.7%)	30 (20.1%)
Yes, n (%)	23 (38.3%)	119 (79.9%)
Marital status		
Divorced, n (%)	4 (6.7%)	2 (1.3%)
Married, n (%)	37 (61.6%)	82 (55.0%)
Single, n (%)	11 (18.3%)	57 (38.3%)
Widowed, n (%)	8 (13.3%)	8 (5.4%)
Alcohol intake		
Alcohol units per week, mean (sd)	6.52 (7.65)	5.33 (7.85)
Familial vs sporadic family history		
FALS, n (%)		91 (61.1%)
SALS, n (%)		58 (38.9%)
C9orf72 repeat expansion status		
Intermediate, n (%)		6 (4.0%)
Negative, n (%)		123 (82.6%)
Positive, n (%)		10 (6.7%)
Missing, n (%)		10 (6.7%)

FALS, Familial ALS; SALS, Sporadic ALS

7.3.2.2. Within the ALS relatives group, 91 were related to an ALS patient with familial ALS, according to the Byrne criteria (351), and 51 were related to an ALS patient with sporadic ALS. Ten unaffected relatives of ALS patients tested positive for the C9orf72 repeat expansion, 123 tested negative, and 6 had an intermediate C9orf72 repeat expansion (i.e., they had between 20 and 30 repeat expansions).

7.3.3. Cognitive screening

7.3.3.1. Cognitive screening was carried out using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). For participants who were unable or did not wish to complete the longer neuropsychological assessment, this was the minimum cognitive assessment performed. Welch's independent samples t-tests were carried out to compare ALS relatives and controls on ECAS total, ALS specific, ALS non-specific, verbal fluency, executive and memory scores. The assumption of normality was violated for language and visuospatial sub-scores; therefore, non-parametric Wilcoxon rank tests were performed.

7.3.3.2. ALS relatives performed significantly worse on ECAS total, ALS specific score, language and verbal fluency score (see table 7.3.). However, after correcting for multiple comparisons, only verbal fluency remained statistically significant (see figure 7.2.).

Table 7.3. ECAS performance in ALS relatives and controls (n = 350).

ECAS score	Control	Relative	t/W	df	p	d/r [95% CI]
ECAS total, mean	112.09	108.58	2.36	272	0.0189	0.29 [0.05, 0.53]
ALS specific, mean	83.11	80.25	2.42	279	0.0162	0.29 [0.05, 0.53]
ALS non-specific, mean	28.96	28.29	1.42	277	0.156	0.17 [-0.06, 0.41]
Language, median ^a	27	27	16474	NA	0.0138	0.13 [0.03, 0.24]
Verbal fluency, mean	19.04	17.25	4.59	333	.0000064*	0.50 [0.28, 0.72]

ECAS score	Control	Relative	t/W	df	p	d/r [95% CI]
Executive, mean	37.56	37.02	0.725	267	0.469	0.09 [-0.15, 0.33]
Memory, mean	17.38	16.74	1.43	285	0.153	0.17 [-0.06, 0.40]
Visuospatial, median ^a	12	12	15009	NA	0.456	0.04 [0.002, 0.14]

* significant after controlling for multiple comparisons

^a assumption of normality violated, non-parametric Wilcoxon rank test performed

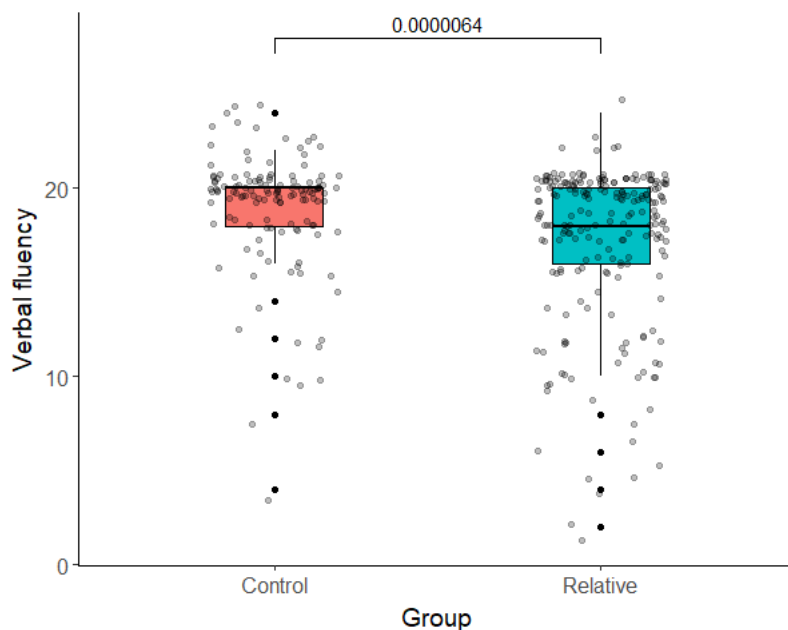


Figure 7.2. Verbal fluency performance in ALS relatives and controls.

7.3.3.3. To explore this deficit in verbal fluency further, the various components of the verbal fluency task (i.e., number of words generated, time to read words, vfi and converted fluency score) were compared using Welch's t-tests and Wilcoxon rank tests. Relatives and controls were compared on both unrestricted (no letter limit) and restricted (4 letter limit)

fluency protocols. Relatives generated significantly fewer words than controls on both unrestricted and restricted protocols (see table 7.4.). As relatives generated fewer words, it is unsurprising that they took significantly less time to read their words. When considering the number of words and the time taken to read them using verbal fluency index (vfi) scores, relatives still performed significantly worse than controls.

Table 7.4. Components of verbal fluency performance: relatives vs controls (n = 334).

Protocol	Verbal fluency component	Controls	Relatives	t/W	df	p	d/r [95% CI]
Unrestricted	Verbal fluency score	9.59	9.06	2.64	192	0.0089	0.38 [0.10, 0.67]
	Verbal fluency index (vfi)	3.40	4.43	-3.71	191	0.0003	-0.54 [-0.83, -0.25]
	Total words	16.72	14.18	3.86	148	0.0002	0.64 [0.30, 0.97]
	Time to read words ^a	11.5	8	9262	NA	.000001 9	0.31 [0.21, 0.43]
Restricted	Verbal fluency score	8.99	8.19	2.49	177	0.0136	0.37 [0.08, 0.67]
	Verbal fluency index (vfi) ^a	5.6	8	6328	NA	0.00029 1	0.21 [0.1, 0.31]
	Total words ^a	9	7	10908	NA	0.00077 5	0.20 [0.08, 0.31]
	Time to read words ^a	7	4	8171	NA	0.00026 7	0.22 [0.09, 0.34]

^a assumption of normality violated; non-parametric Wilcoxon rank test performed

7.3.3.4. One-way analyses of variance (ANOVAs) were carried out to examine the effect of family history on ECAS performance. This compared ECAS performance in relatives of familial ALS (FALS) patients, relatives of sporadic ALS (SALS) patients and controls. In cases where the assumptions of ANOVA were violated, non-parametric Kruskal-Wallis tests were carried out.

7.3.3.5. Significant main effects were observed for ECAS total score $F(2,342) = 4.47$, $p < .05$, $w^2 = 0.02$, ALS specific score, $F(2,342) = 4.657$, $p < .05$, $w^2 = 0.02$; and verbal fluency score, $H(2) = 31.81$, $p < .001$. However, after controlling for multiple comparisons, only the main effect for verbal fluency remained significant. Bonferroni-Holm post-hoc tests were subsequently carried out to contrast FALS relatives, SALS relatives and controls directly on verbal fluency scores.

7.3.3.6. Bonferroni-Holm post-hoc tests indicated that FALS relatives scored significantly worse than controls on verbal fluency score (See figure 7.3.). No significant differences were observed between FALS and SALS relatives; or between SALS and controls.

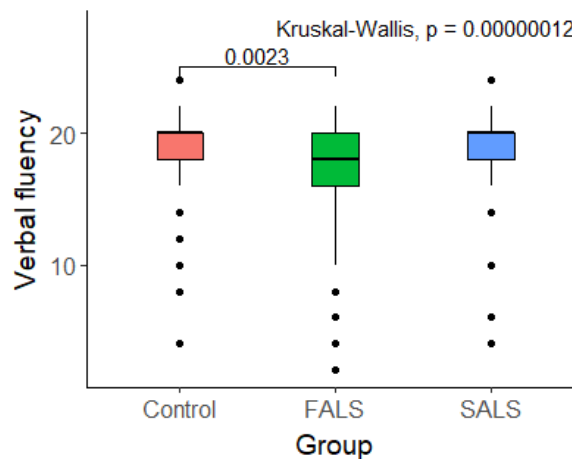


Figure 7.3. ECAS verbal fluency score in FALS relatives, SALS relatives and controls.

7.3.3.7. Finally, the effect of the C9orf72 repeat expansion on ECAS performance was examined by comparing C9orf72 positive and C9orf72 negative ALS relatives. Welch's independent t-tests were carried out for ECAS total, ALS specific, ALS non-specific, executive and memory scores. Wilcoxon rank tests were computed for language, verbal fluency and visuospatial sub-scores as data were non-parametric. No significant differences were found between C9orf72 positive and C9orf72 negative ALS relatives on any ECAS score.

7.3.4. Intellectual functioning

7.3.4.1. Current intellectual functioning was assessed using the Wechsler Abbreviated Scale of Intelligence- 2nd edition (WASI-II). The vocabulary and matrix reasoning subtests of the WASI-II were carried out. Performance was converted to t-scores using age derived norms from the test manual. Vocabulary and Matrix Reasoning t-scores were then summed and converted to Full-Scale IQ-2 (FSIQ-2) standard scores.

7.3.4.2. Welch's independent samples t-tests indicated that relatives had significantly lower vocabulary score, $t(94.34) = 2.18$, $p < .05$, $d = 0.45$ [95% CI: 0.04, 0.86]; matrix reasoning score, $t(96.96) = 2.62$, $p < .05$, $d = 0.53$ [95% CI: 0.13, 0.94]; and FSIQ-2 score, $t(94.03) = 2.62$, $p < .05$, $d = 0.54$ [95% CI: 0.13, 0.95].

7.3.4.3. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls on WASI-II scores. Significant main effects were observed for FSIQ-2, $F(2,199) = 3.94$, $p < .05$, $w^2 = 0.03$; and vocabulary score, $F(2,204) = 3.5$, $p < .05$, $w^2 = 0.02$, after controlling for multiple comparisons.

7.3.4.4. Post hoc Bonferroni-Holm comparisons indicated that FALS relatives scored significantly lower than controls ($p < .05$) on both FSIQ-2 and vocabulary scores (see figure 7.4.). No significant differences were observed between FALS relatives and SALS relatives, or between SALS relatives and controls.

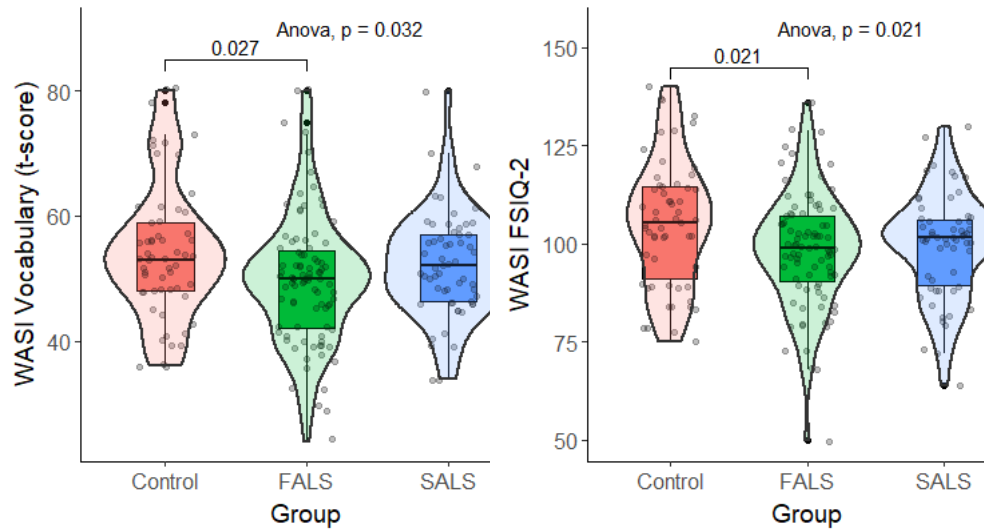


Figure 7.4. WASI-II Vocabulary (a) and Full-Scale IQ (FSIQ) score in FALS relatives, SALS relatives and controls.

7.3.4.5. Welch's independent samples t-tests found no significant differences between C9orf72 positive and C9orf72 negative relatives on any WASI-II score.

7.3.5. Executive functioning

7.3.5.1. Verbal fluency

7.3.5.1.1. Verbal fluency performance was assessed using the FAS test, restricted fluency and semantic fluency. This verbal fluency protocol is more in-depth than the ECAS verbal fluency task discussed earlier. Phonemic fluency is examined using three letters F, A and S, with a minute provided for each. The total words generated in the three minutes are summed to give a FAS total score. This was converted to z-scores using age and education derived norms. A restricted protocol is then carried out, where participants are limited to 4 letter words beginning with C. No normative data were

available to generate z-scores for this restricted protocol, therefore raw vfi scores were used in this analysis. Lastly, semantic fluency was assessed, where participants must name as many animals as possible in a minute. Raw scores were converted to z-scores using age and gender normative data.

7.3.5.1.2. Welch's independent samples t-tests indicated that ALS relatives performed significantly worse than controls on FAS total score, $t(88.77) = 3.79$, $p < .001$, $d = 0.81$ [95% CI: 0.37, 1.24], after controlling for multiple comparisons. No significant difference was observed between relatives and controls on restricted or semantic fluency paradigms.

7.3.5.1.3. One-way ANOVAs were carried out to examine the effect of family history on verbal fluency scores, i.e., comparing FALS relatives, SALS relatives, and controls. A significant main effect was observed for FAS total score, $F(2,198) = 12.51$, $p < .001$, $w^2 = 0.1$ and restricted fluency, $F(2,204) = 4.39$, $p < .05$, $w^2 = 0.03$ (see figure 7.5). No significant main effect was observed for semantic fluency.

7.3.5.1.4. Post-hoc Bonferroni-Holm comparisons found that FALS relatives scored significantly worse than controls ($p < .001$), and SALS relatives ($p < .05$) on FAS total score. For restricted verbal fluency, FALS relatives scored significantly worse than SALS relatives (for vfi, higher scores indicate poorer performance). No other post-hoc comparisons were significant.

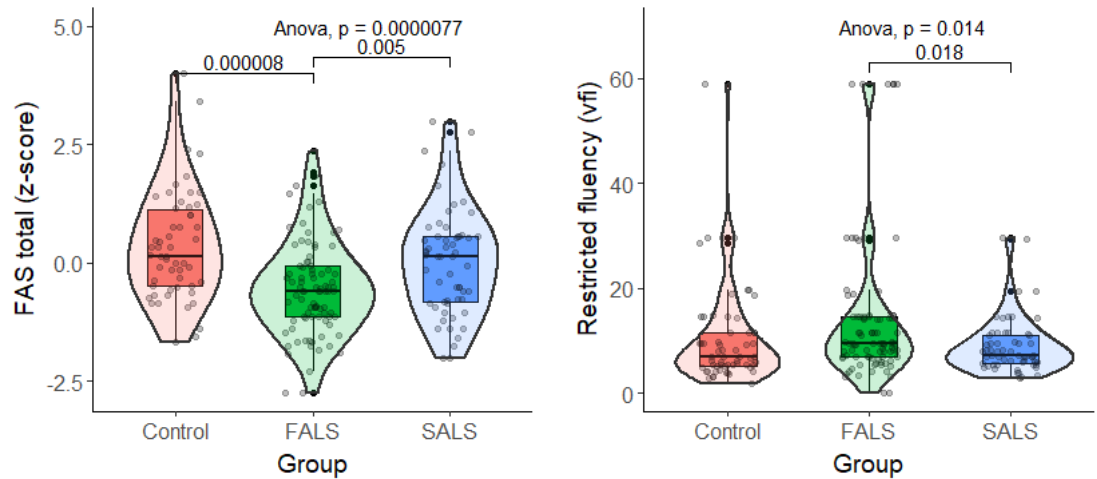


Figure 7.5. FAS total z-score and restricted verbal fluency index score in FALS relatives, SALS relatives and controls. Note: lower vfi indicates better performance.

7.3.5.1.5. Welch's independent samples t-tests were carried out to examine the effect of C9orf72 status on verbal fluency performance. No significant difference was observed between C9orf72 positive and C9orf72 negative relatives on FAS total or restricted fluency scores. However, C9orf72 negative relatives performed significantly worse than C9orf72 positive relatives on semantic fluency, $t(21.23) = -2.87$, $p < .01$, $d = -1.25$ [95% CI: -2.16, -0.31] (see figure 7.6.).

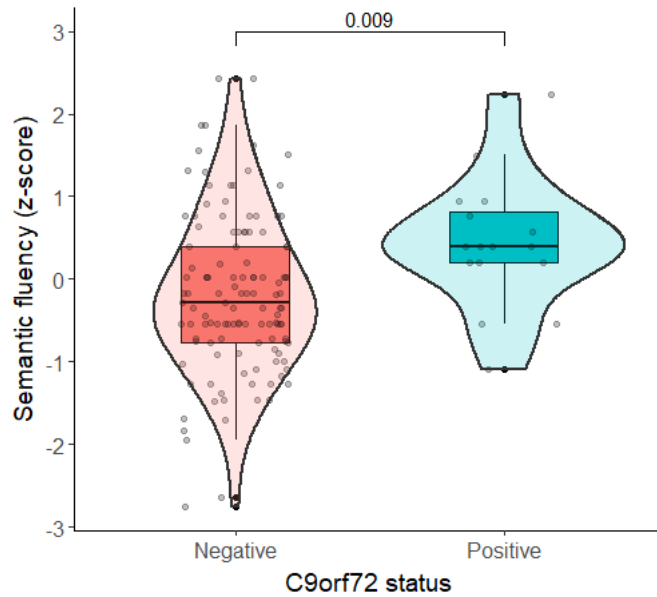


Figure 7.6. Semantic fluency in C9orf72 positive and C9orf72 negative relatives.

7.3.5.2. Inhibition/switching

7.3.5.2.1. Inhibition and switching performance were measured using the colour-word interference test (CWIT). This task consists of 4 subtests: colour naming, word naming, inhibition and inhibition/switching. Raw scores were converted to scaled scores using age derived norms. Welch's independent t-tests were carried out to compare performance on these subtests in ALS relatives and controls. Performance was measured in terms of the time taken to complete each task, and in terms of the number of errors made for inhibition and inhibition/switching subtests.

7.3.5.2.2. No significant differences were observed in terms of the time taken to complete each CWIT subtask. However, ALS relatives had significantly more errors on the inhibition subtest, $t(158.6) = 3.08$, $p < .005$, $d = 0.49$ [95% CI: 0.17, 0.80], even when controlling for multiple

comparisons. No significant deficit was observed for CWIT switching/inhibition task.

7.3.5.2.3. One-way ANOVAs were performed to examine the effect of family history on CWIT performance, i.e., comparing FALS relatives, SALS relatives and controls. A significant main effect was only observed for the number of errors on the CWIT inhibition task, $F(2,197) = 3.69$, $p < .05$, $w^2 = 0.03$. Post-hoc Bonferroni-Holm comparisons indicated that SALS relatives performed significantly worse than controls ($p < .05$). No other post-hoc comparisons were significant (See figure 7.7.).

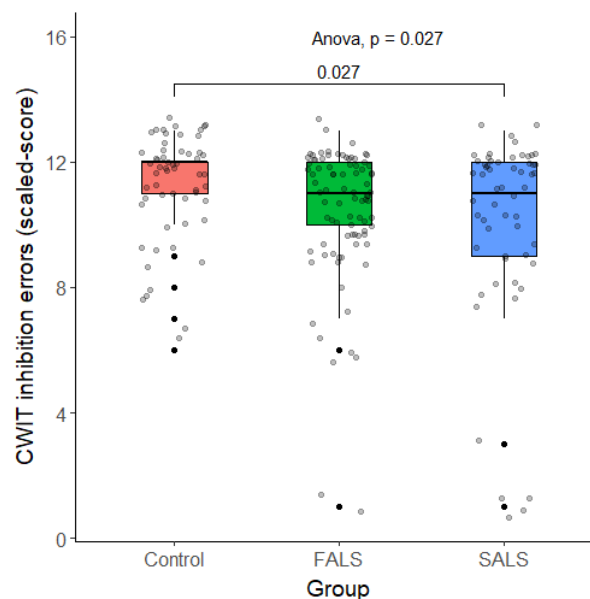


Figure 7.7. CWIT inhibition errors scaled in FALS relatives, SALS relatives and controls. Note: lower scales scores mean's poorer performance. Also note the presence of 2 groups of outliers in the FALS and SALS groups with scaled scores less than 4.

7.3.5.2.4. Figure 7.7. indicated 2 groups of outliers in both FALS and SALS groups ($n = 7$). The demographic and clinical information of these individuals was explored to examine why they might have performed so

poorly. Aside from 2 individuals, all other participants were from different families, suggesting this was not driven by one family. All participants were C9orf72 negative. All had moderate levels of education and spanned a wide age range (32-62). Ultimately, there were no obvious factors that account for why this subgroup had so many inhibition errors.

7.3.5.2.5. CWIT performance was compared between C9orf72 positive and negative relatives. C9orf72 negative participants took significantly longer time on the switching subtask, $t(26.36) = -2.32$, $p < .05$, $d = -0.90$ [95% CI: -1.70, -0.09]; however, this did not survive correction for multiple comparisons.

7.3.5.3. Working memory

7.3.5.3.1. Working memory was measured using the digit span test from the D-KEFS. This includes digit span forward, backwards and sequential subtasks. Performance was quantified in terms of the longest span participants got correct, which was converted to z-scores using age derived normative data.

7.3.5.3.2. Welch's independent samples t-tests indicated that ALS relatives performed significantly worse than controls on the digit span backwards subtask, $t(109) = 4.19$, $p < .001$, $d = 0.8$ [95% CI: 0.41, 1.19]. No significant difference was observed on digit span forwards or sequential subtasks.

7.3.5.3.3. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls. A significant main effect was observed for digit span backwards span, $F(2,202) = 9.31$, $P < .001$, $\eta^2 = 0.08$. No significant

main effect was observed for forward or sequential span. Post-hoc Bonferroni-Holm comparisons revealed that FALS relatives and SALS relatives both performed significantly worse than controls (both $p < .05$). No significant difference was observed between FALS and SALS relatives (see figure 7.8.).

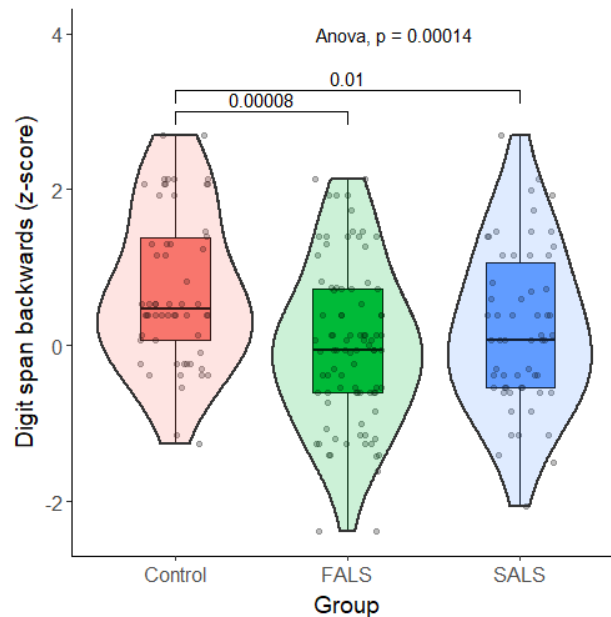


Figure 7.8. Backwards digit span in FALS relatives SALS relatives and controls.

7.3.5.3.4. Welch's t-tests and Wilcoxon rank tests found no significant difference between C9orf72 positive relatives and C9orf72 negative relatives on digit span forward, backwards or sequential spans.

7.3.5.4. Sustained attention

7.3.5.4.1. Sustained attention was measured using the sustained attention to response task (SART). In this task, participants are shown a series of numbers on a computer screen. They are instructed to press the space bar as quickly as possible when they see any number, except the number 3.

Participants were assessed in terms of how many omission errors (failing to press the space bar when they should have) and commission errors (pressing the space bar when they shouldn't have). Participants can also make anticipation errors if they respond too soon (within 200ms of stimuli presentation). Participants were also compared in terms of their mean reaction time. All scores were converted to z-scores using published healthy control data. However, these norms were not stratified by age.

7.3.5.4.2. Welch's independent samples t-tests found no significant differences between relatives and controls in terms of the number of omission, commission or anticipation errors they made. However, ALS relatives had a significantly faster mean reaction time than controls, $t(76.21) = -2.87$, $p < .01$, $d = -0.66$ [95% CI: -1.12, -0.20].

7.3.5.4.3. One-way ANOVAs were carried out to examine the effect of family history on SART performance. No significant main effect was observed for omission, commission or anticipation errors. However, a significant main effect was found for mean reaction time, $F(2,78) = 4.47$, $p < .05$, $w^2 = 0.08$. Bonferroni-Holm post hoc comparisons revealed that SALS relatives were significantly faster than both FALS relatives and controls on overall mean reaction time ($p < .05$, see figure 7.9.).

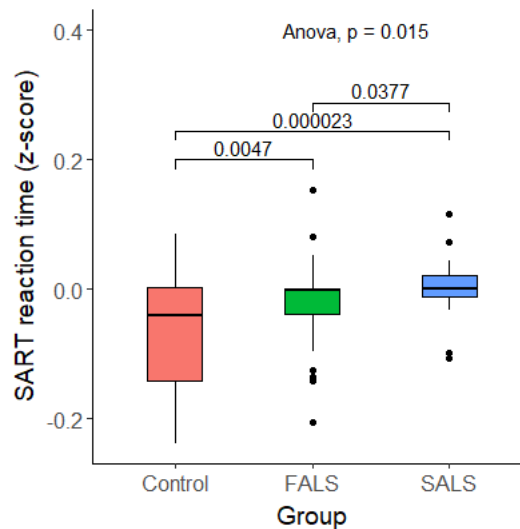


Figure 7.9. SART mean reaction time in FALS relatives, SALS relatives and controls.

7.3.5.4.4. Not enough C9orf72 positive participants had complete SART data to allow for comparison with the C9orf72 negative group.

7.3.5.5. Emotion based decision making

7.3.5.5.1. Emotion based decision making was assessed using the Iowa Gambling Task (IGT). This computerized card game test requires a person to make risk-reward decisions in picking from 4 decks of cards. To win money in the game, the participant must learn to favor low reward - low risk decks over high risk - high reward decks. Total IGT score was converted to t-scores using normative data provided using the IGT software. The IGT program also splits the task into 5 sections, providing a measure of how participants adjust their decisions over time.

7.3.5.5.2. Welch's independent samples t-tests indicated that relatives performed significantly worse than controls on IGT total, $t(91.78) = 2.36$, $p < .05$, $d = 0.49$ [95% CI: 0.08, 0.91] (see figure 7.10.a). When the 5 IGT

sub-scores were examined, there were no significant differences between relatives and controls for sections 1, 2, 3 or 4. However, at section 5, relatives performed significantly worse than controls, $t(96.65) = 2.93$, $p < .01$, $d = 0.60$ [95% CI: 0.19, 1.00]. This suggests that over time, controls are learning which decks to avoid while the relatives do not (see figure 7.10.b).

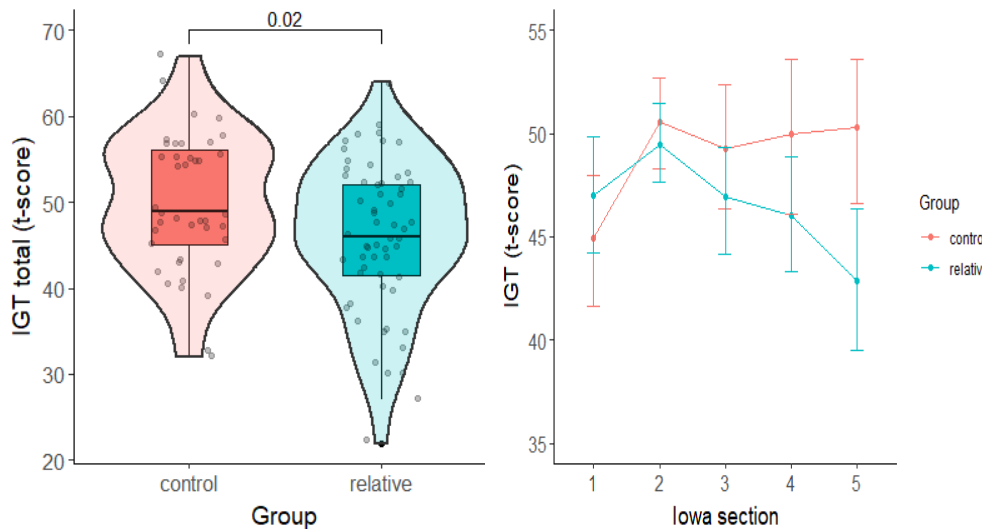


Figure 7.10. IGT total (a) and IGT performance over time (b) in relatives and controls.

7.3.5.5.3. One-way ANOVAs were carried out to compare SALS and FALS relatives to controls. No significant main effect was observed for IGT total score, or for sections 1 to 4. A significant main effect was observed for IGT section 5, $F(2,97) = 3.9$, $p < .05$, $w^2 = 0.05$. However, this did not survive correction for multiple comparisons.

7.3.5.5.4. Not enough C9orf72 positive participants had complete IGT data to allow for comparison with the C9orf72 negative group.

7.3.6. Language (confrontation naming)

7.3.6.1. Language functioning was assessed using the Boston Naming Test (BNT) – 30 item version, a short test of confrontational naming. BNT performance is quantified using 3 scores: total correct with no cue (spontaneous score), total correct after a semantic cue (semantic score), and total correct after a phonemic cue (phonemic score). Raw scores were converted to z-scores using age derived Irish normative data.

7.3.6.2. Welch's independent samples t-test indicated that relatives performed significantly worse than controls on all BNT scores: spontaneous, $t(200.64) = 6.42$, $p < .001$, $d = 0.91$ [95% CI: 0.61, 1.20]; semantic, $t(192.63) = 6.03$, $p < .001$, $d = 0.87$ [95% CI: 0.57, 1.16]; and phonemic score, $t(201.1) = 6.01$, $p < .001$, $d = 0.85$ [95% CI: 0.56, 1.13].

7.3.6.3. Kruskal-Wallis tests were carried out to examine the effect of family history of ALS on BNT performance. Again, significant main effects were found for all BNT scores: spontaneous, $H(2) = 28.71$, $p < .001$; semantic, $H(2) = 24.25$, $p < .001$; and phonemic $H(2) = 27.92$, $p < .001$. Non-parametric post-hoc comparisons indicated significant differences between FALS relatives and controls ($p < .05$) and between SALS relatives and controls ($p < .05$) on all BNT scores. No significant differences were observed between FALS and SALS relatives on all BNT scores (see figure 7.11.).

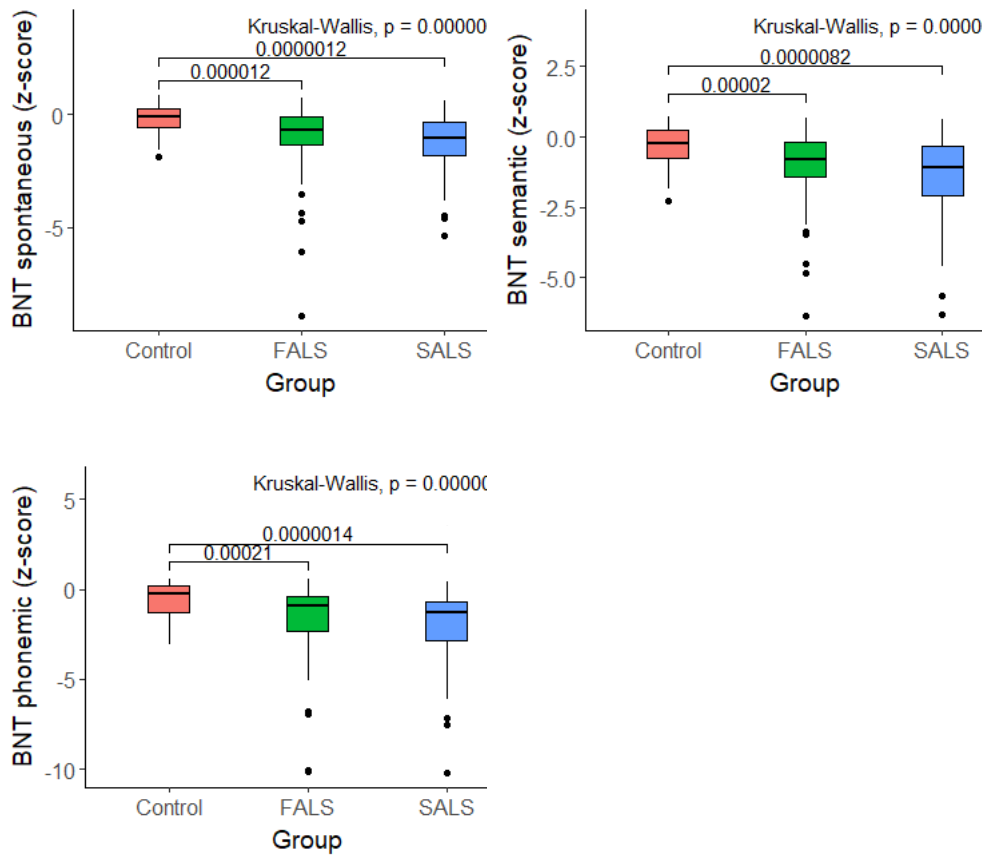


Figure 7.11. BNT spontaneous, semantic and phonemic scores in FALS relatives, SALS relatives and controls.

7.3.6.4. Welch's independent samples t-tests indicated significant differences between C9orf72 positive and C9orf72 negative relatives on BNT performance (see figure 7.12.). Participants who were C9orf72 negative scored significantly worse on BNT spontaneous, $t(25.8) = -2.38$, $p < .05$, $d = -0.93$ [95% CI: -1.74, -0.11]; semantic, $t(27.92) = -2.91$, $p < .01$, $d = -1.1$ [95% CI: -1.89, -0.30]; and phonemic score, $t(41.41) = -4.59$, $p < .001$, $d = -1.43$ [95% CI: -2.1, -0.74].

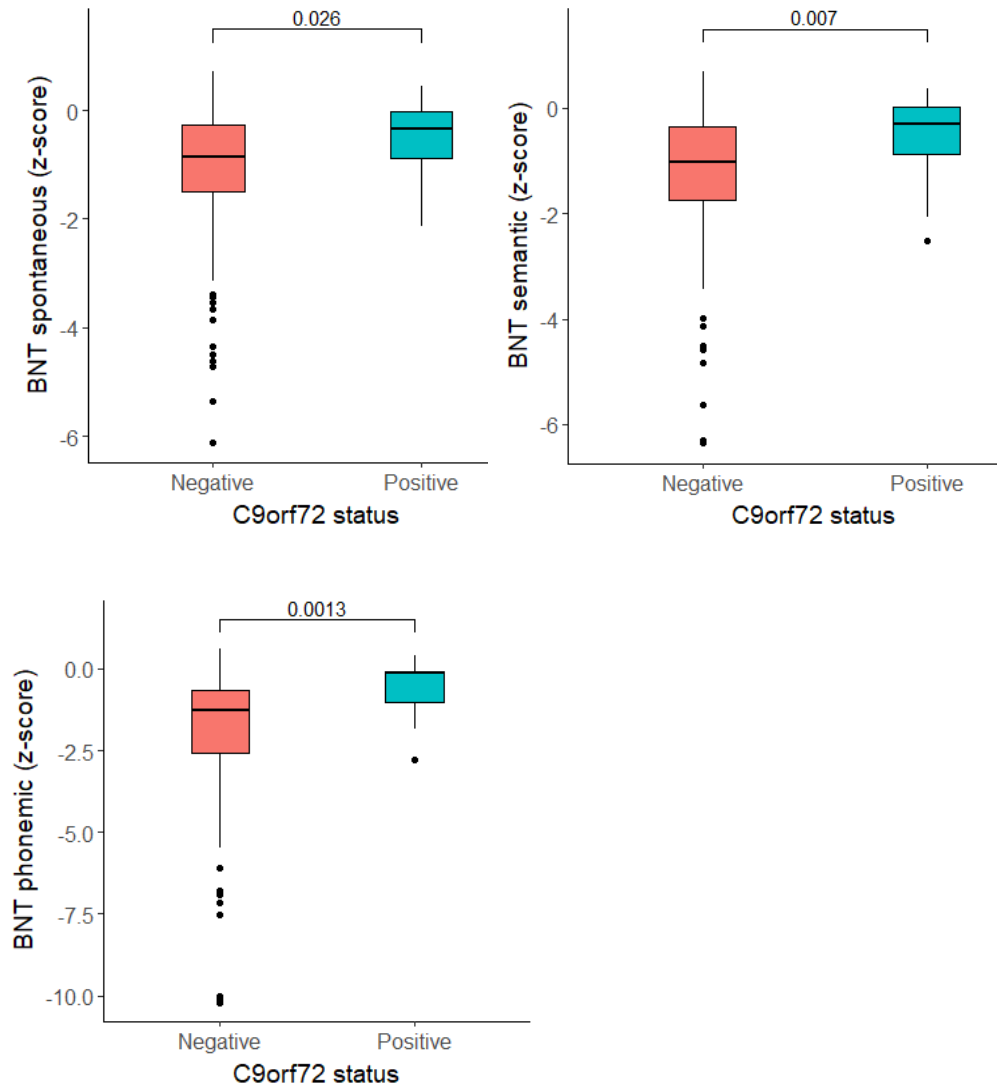


Figure 7.12. BNT spontaneous, semantic and phonemic performance in C9orf72 positive and C9orf72 negative relatives.

7.3.7. Memory

7.3.7.1. Verbal memory

7.3.7.1.1. Verbal list learning was assessed using the Rey Auditory Verbal Learning Test (RAVLT). For this task, participants listen to a list of 15 words and are asked to recall them. The list is called out 5 times, and the participants must immediately recall the list after each trial. Participants are

then read a different ‘distractor’ list. After this they are asked to recall the first list again. Finally, after 20 minutes, they are asked to recall the first list. RAVLT performance was assessed in terms of the total number of immediately recalled words, delayed recall score and recognition score, all of which were standardized using gender and age matched normative data.

7.3.7.1.2. Welch’s independent samples t-tests indicated that ALS relatives performed significantly worse than controls on RAVLT immediate, $t(97.52) = 3.31, p < .01, d = 0.67$ [95%CI: 0.26, 1.08]; and RAVLT delayed score, $t(88.11) = 2.52, p < .05, d = 0.54$ [95%CI: 0.11, 0.96]. No significant difference was observed on RAVLT recognition performance.

7.3.7.1.3. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls. A significant main effect was observed for RAVLT immediate, $F(2,199) = 5.93, p < .01, w^2 = 0.05$; and RAVLT delayed recall, $F(2,200) = 4.15, p < .05, w^2 = 0.03$. Post hoc Bonferroni-Holm comparisons indicated that for RAVLT total, both FALS and SALS relatives scored significantly worse than controls ($p < .05$). For RAVLT delayed, only FALS relatives scored significantly worse than controls ($p < .05$) (see figure 7.13.).

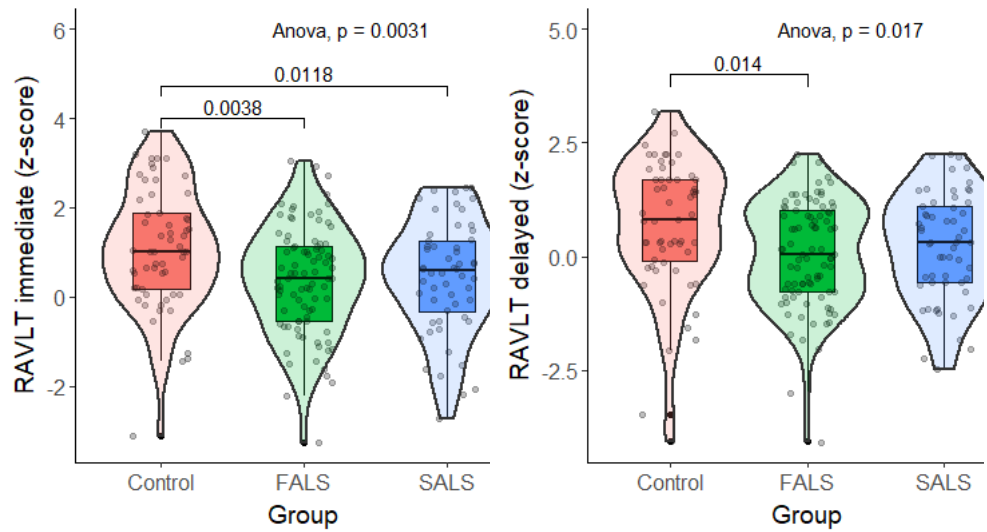


Figure 7.13. RAVLT immediate and delayed recall in FALS relatives, SALS relatives and healthy controls.

7.3.7.1.4. No significant differences were observed between C9orf72 positive and C9orf72 negative relatives on all RAVLT scores.

7.3.7.1.5. To further investigate the deficit in list recall (immediate and delayed), the slope of learning over time was examined (see figure 7.14). Relatives performed worse than controls on from the beginning of the task, however the rate of learning over time was similar.

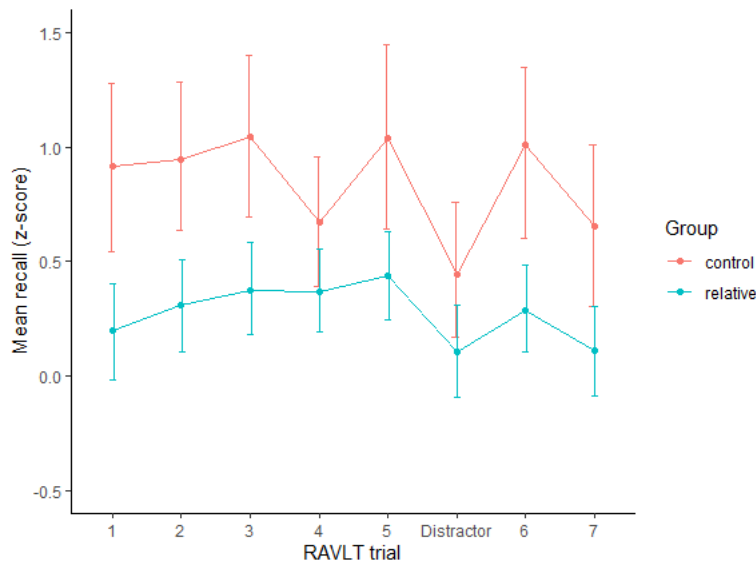


Figure 7.14. RAVLT learning slope for ALS relatives and healthy controls.

7.3.7.1.6. Episodic verbal memory was assessed using the logical memory subtest of the Wechsler Memory Scale (3rd edition). This requires an individual to listen to 2 short stories and then recall them immediately (immediate recall), and after 20 minutes (delayed recall). Finally, participants are asked a series of questions about the stories (recognition). Scores were converted to z-scores using age matched normative data.

7.3.7.1.7. Relatives and controls did not differ on immediate recall or recognition. However, relatives performed significantly worse than controls on delayed recall, $t(97.85) = 3.48$, $p < .001$, $d = 0.7$ [95% CI: 0.29, 1.11].

7.3.7.1.8. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls. No significant main effect was observed for immediate or recognition memory. A significant main effect was observed for delayed recall, $F(2,193) = 6.29$, $p < .01$, $w^2 = 0.05$. Post hoc Bonferroni-

Holm comparisons revealed that both FALS and SALS relatives both performed significantly worse than controls ($P < .01$ in both cases, see figure 7.15).

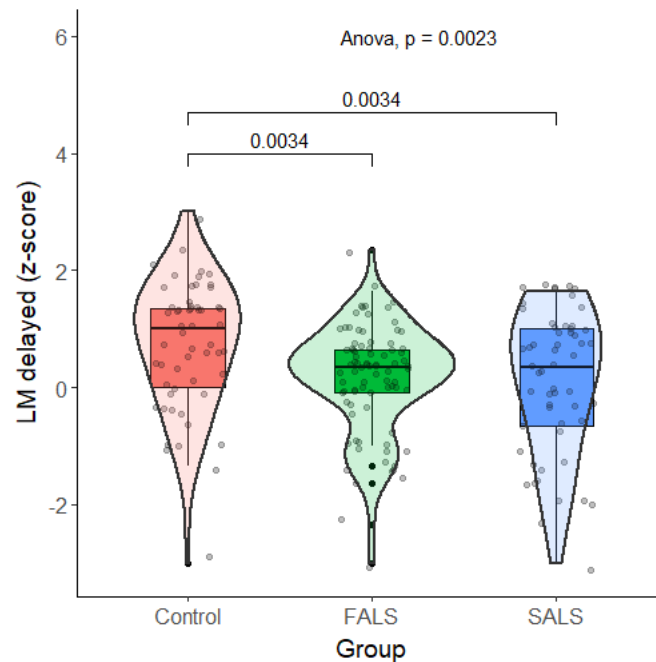


Figure 7.15. Logical memory delayed performance in FALS relatives, SALS relatives and controls.

7.3.7.1.9. No significant differences were observed between C9orf72 positive and C9orf72 negative relatives on any logical memory scores.

7.3.7.2. Visuospatial memory

7.3.7.2.1. Visuospatial memory was measured using the Rey-Osterrieth Complex Figure Test (RCFT). Participants were shown a complex line drawing and asked to copy it. The figure is then withdrawn, and the participant is required to draw the same figure from memory (immediate recall). After 20 minutes, they were again asked to draw the figure (delayed

recall). Finally, they are shown 24 smaller figures and asked if they are a component of the original figure or not (recognition). Raw scores were converted to z-scores using age derived normative data.

7.3.7.2.2. No significant difference was observed between ALS relatives and controls on RCFT copy accuracy. However, controls spent significantly longer time copying the figure, $t(143.8) = -3.36$, $p < .01$, $d = -0.56$ [95% CI: -0.89, -0.23]. Relatives scored significantly worse than controls on RCFT immediate recall, $t(90.83) = 3.99$, $p < .001$, $d = 0.84$ [95% CI: 0.41, 1.26]; delayed recall, $W = 4843$, $p < .01$, $r = 0.199$ [95% CI: 0.06, 0.33]; and recognition score, $t(118.45) = 2.85$, $p < .01$, $d = 0.52$ [95% CI: 0.16, 0.89].

7.3.7.2.3. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls on RCFT performance. After controlling for multiple comparisons, no significant main effect was observed for RCFT copy, copy time, delayed or recognition score. A significant main effect was observed on RCFT immediate recall, $F(2,128) = 10.68$, $p < .001$. Post-hoc Bonferroni-Holm comparisons revealed that for RCFT immediate score, controls scored the highest, followed by FALS relatives, and the SALS relatives (see figure 7.16).

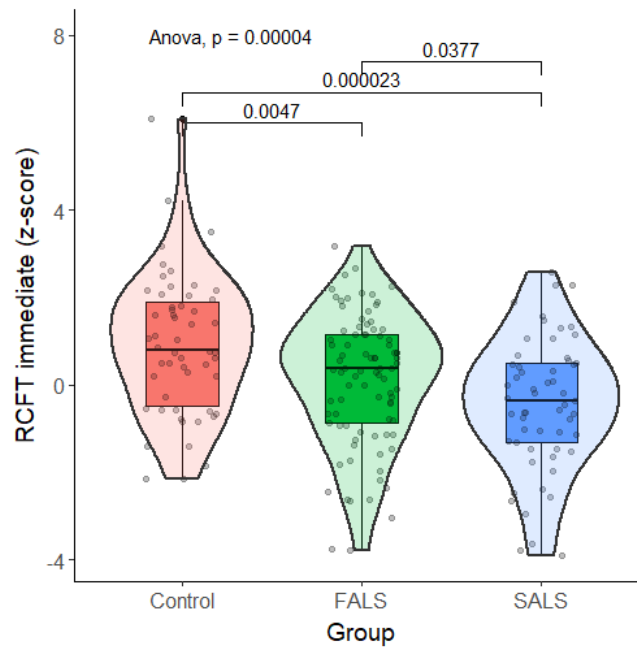


Figure 7.16. RCFT immediate recall in FALS relatives, SALS relatives and healthy controls.

7.3.7.2.4. No significant differences were observed between C9orf72 positive and C9orf72 negative relatives on all RCFT scores.

7.3.8. Social cognition

7.3.8.1. Social cognition was examined using the Reading the Mind in the Eyes Test (RMET). This is a short test that requires the participant to identify the emotional states of 32 black and white face pictures. RMET raw score (total correct) was converted to z-scores using published Irish control data. However, this data was not stratified by age, education or gender.

7.3.8.2. Welch's independent samples t-test indicated no significant difference between relatives and controls on RMET performance. A one-way ANOVA found no significant main effect comparing FALS, SALS

relatives and controls. Wilcox rank test found no significant difference between C9orf72 positive and C9orf72 negative relatives.

7.4. Summary of findings

7.8.1. First- and second-degree relatives of ALS patients performed significantly worse than controls across a range of neuropsychological tests, including WASI-II, verbal fluency tasks (both ECAS and the FAS test), the CWIT inhibition subtask, digit span backwards, SART reaction time, the IGT, the BNT, RAVLT immediate and delayed recall, logical memory delayed recall and RCFT delayed recall.

7.8.2. A family history of ALS had a significant effect on performance on certain tasks. Relatives of FALS patients showed significantly worse performance on verbal fluency than relatives of SALS patients. Conversely, SALS relatives performed significantly worse than FALS relatives on RCFT immediate recall.

7.8.3. Analysis of C9orf72 subgroups revealed few differences between C9orf72 positive and negative relatives, however, this was limited by the small number of C9orf72 positive relatives with full neuropsychological data. Semantic verbal fluency score was the only test where a significant difference was observed, with C9orf72 negative relatives scoring significantly worse than C9orf72 positive relatives.

7.5. Discussion

7.5.1. The results of this study show that relatives of ALS patients show a range of cognitive deficits, when compared to healthy controls with no

family history of ALS. These deficits were present across multiple cognitive domains, including IQ, executive functioning, language and memory. In chapter 3, ALS relatives were hypothesized to have deficits in executive functioning and language tasks, as these domains are commonly affected in ALS patients (59,226). The multiple deficits on memory and IQ tasks were not expected, as these functions are less commonly affected in ALS (33).

7.5.2. Despite similar levels of education, ALS relatives had significantly lower intelligence than controls. This was evident in both verbal and non-verbal intelligence domains. While not commonly reported, verbal IQ deficits have been observed in ALS patients (32), though usually with small effect sizes. IQ was not assessed in the two studies of pre-symptomatic ALS risk gene carriers (151,345). Furthermore, these studies excluded individuals with low scores on screening measures, likely biasing the population estimates. If ALS relatives have significant deficits in IQ, as was observed here, this may explain why ALS relatives performed worse than controls across such a wide range of tasks. In chapter 9, IQ deficits and their impact on executive, language and memory tests deficits in ALS relatives are examined.

7.5.3. One of the most robust findings observed in this study was that ALS relatives had significant impairments on phonemic verbal fluency. The reliability of this finding is evidenced by phonemic fluency deficits on both the ECAS fluency subtests and the FAS test. The effect sizes of these deficits were large, and clustered in relatives of FALS patients. Phonemic fluency deficits were present in both C9orf72 positive and negative ALS

relatives, implicating the involvement of both known and currently unknown gene variants.

7.5.4. A deficit was not observed for the restricted fluency paradigm, possibly due to the lack of age specific norms for this task. There was also no deficit on semantic fluency, despite the fact that these deficits are often impaired in patients (226,353). The fact that phonemic fluency is affected, while semantic fluency is spared, would suggest that this endophenotype is underpinned by frontal and prefrontal lobe involvement, rather than medial-temporal lobe involvement (354).

7.5.5. While ALS relatives as a whole did not differ from controls in terms of semantic verbal fluency, C9orf72 negative relatives performed significantly worse than C9orf72 positive relatives. The directionality of this result is contradicts previous studies of asymptomatic C9orf72 carriers (151,345). While limited in terms of sample size, this finding suggests the presence of a non-C9orf72 endophenotype, characterized by impaired semantic fluency and medial temporal involvement. Semantic fluency deficits have been observed in unaffected siblings and parents of schizophrenia patients (347,355,356), and clusters to a greater extent in individuals with a stronger family history. It is possible that semantic fluency deficits are an endophenotype of non-C9orf72 mediated ALS and schizophrenia, accounting for the known genetic correlation between ALS and schizophrenia (136).

7.5.6. Phonemic fluency deficits are well established as one of the most sensitive markers of impairment in ALS, associated with impaired activation

of middle and inferior frontal gyri, the anterior cingulate gyrus and reduced activity in parietal and temporal lobes (357). The identification of similar phonemic fluency deficits in unaffected relatives, that clusters in those at higher risk, provides good evidence for its suitability as a candidate endophenotype.

7.5.7. Analysis of the CWIT revealed that ALS relatives took the same amount of time to complete each subtask as controls, however, they made significantly more inhibition errors. In contrast to the verbal fluency deficits discussed above, inhibition errors appeared to cluster in relatives of SALS patients. Inhibition deficits are typically associated with dysfunction of the dorsolateral prefrontal cortex and the anterior cingulate cortex. Of note, ALS relatives did not make significantly more errors than controls on the switching/inhibition subtest of the CWIT. This may imply a pure inhibition endophenotype of SALS or could simply be because the inhibition/switching subtask is administered after the inhibition task, resulting in practice effects that obscure any effect.

7.5.8. ALS relatives showed normal digit span forward, but abnormal digit span backwards. This would suggest that general attention and the phonological loop is preserved, but verbal working memory is impaired (358). This is corroborated by SART performance, where ALS relatives made a similar number of omission, commission and anticipation errors as controls. While relatives had a significantly faster mean reaction time on the SART, this is likely due to the younger age profile of this group and the lack of age-specific norms for this task. As observed on the CWIT, the final

subtask of the digit span, sequential span, showed no significant difference between relatives and controls despite its similar demands on working memory. Again, this may be due to procedural learning that occurs over the course of the digit span protocol.

7.5.9. ALS relatives had significantly worse performance on the IGT, suggestive of emotion-based decision-making dysfunction. Analysis of decisions over time on the IGT revealed that relatives were indistinguishable from controls up until the final stage of the task, i.e., controls made better decisions as the task went on while ALS relatives made worse decisions. Poorer IGT performance is typically associated with amygdala, orbitofrontal and ventromedial prefrontal cortex dysfunction (183).

7.5.10. IGT deficits in ALS relatives can also be viewed from the somatic marker hypothesis (310). Somatic markers are feelings in the body that are associated with an emotion (e.g., racing heartbeat when anxious, nausea when disgusted) that drive decision making. Impaired performance on the IGT may be due to an inability to form these somatic markers.

7.5.11. As well as verbal fluency, confrontational naming deficits were particularly pronounced in ALS relatives. ALS relatives performed worse on all sub-scores of the BNT, i.e., spontaneous, semantic and phonemic scores. These deficits were present in both FALS relatives and SALS relative and were more pronounced in C9orf72 negative individuals (large effect sizes ranging from $d = -0.93$ - -1.43). Previous pre-symptomatic studies found no such deficits on confrontation naming in C9orf72 negative relatives.

However, as stated, these studies had strict exclusion criteria that may have

limited their results. Furthermore, the Lee et al. (345) study is limited by the inclusion of non-C9orf72 family members in their control group along with members of the general population.

7.5.12. ALS relatives performed significantly worse than controls on immediate and delayed verbal and visuospatial memory. Verbal and visuospatial deficits have been reported in ALS, however, these are often attributed to executive dysfunction (359), resulting in encoding difficulty. Analysis of the slope of learning on the RAVLT supports this argument, as ALS relatives recalled fewer words than controls from the beginning of the task but showed a similar rate of learning.

7.5.13. Given the vast range of tests that ALS relatives displayed deficits on, it is surprising that there was no significant difference on social cognition performance. However, this may be due to the lack of age-specific norms for the RMET. Social cognitive deficits are commonly observed in both ALS and FTD (228). As with memory, the extent to which these deficits are attributable to executive dysfunction remains unclear (360).

7.5.14. The effect sizes observed in this study were generally in line with what was expected, as outlined in the sample size calculation (see Chapter 6.9.2. for detail). Studies of pre-symptomatic ALS risk gene carriers and of unaffected relatives of schizophrenia patients reported median effect sizes of ~ 0.7 (151,345–347). The largest effect sizes observed in this study were for verbal fluency ($d = 0.81$) and confrontational naming ($d = 0.91$).

Endophenotypes are usually more subtle in unaffected relatives than in patients (i.e., they show an intermediate level of deficits). However, the

magnitude of these deficits in this study were equal, if not greater, than those observed in patients (32).

7.5.15. The moderate to large effect sizes observed in this study suggests that these deficits may have clinical significance. For example, Cohen states that effect sizes of 0.80 or greater are grossly perceptible (352). However, it is important to note that effect sizes are measures of group level differences and can vary from population to population. For example, in the two of pre-symptomatic C9orf72 carriers, Lule et al. (151) report an effect size of $d = 0.76$, while a similar study by Lee et al. (345), report a non-significant effect size of $d = 0.11$. Future research of this population is needed to establish the true effect size of these deficits before their clinical significance can be determined.

7.6. Limitations

7.6.1. Due to the Covid-19 pandemic, control recruitment was curtailed, resulting in a relatively small, older control group. While the utilization of age-specific norms reduced the confounding effect of age for most tests, some tests, such as the SART, restricted verbal fluency and the RMET, did not have age stratified norms available. For tests where norms were available, these norms were typically developed in American populations, and may not be optimal for an Irish cohort.

7.6.2. The neuropsychological assessment was largely tailored towards executive functioning, due to its relevance in ALS. However, confrontational naming was the most impaired function in unaffected relatives. A more

detailed language assessment may have revealed further deficits in ALS relatives.

7.6.3. Despite the large sample of ALS relatives, relatively few were C9orf72 positive, limiting the power to detect differences. In contrast to previous studies, C9orf72 negative relatives appeared to have worse performance on semantic fluency and confrontational naming than C9orf72 positive. Further research on C9orf72 positive and C9orf72 negative relatives will be needed to validate these findings. These studies should be mindful of screening individuals with low cognitive screening scores and to not mix non-C9orf72 relatives with members of the general population in with their control groups.

8. Chapter 8. Results 2: Neuropsychiatric Endophenotypes in Unaffected Relatives of ALS patients

8.1. Introduction

8.1.1. This chapter examined aim 4 of this thesis, which was to identify candidate neuropsychiatric endophenotypes of ALS. To do this, first and second-degree relatives of ALS patients were compared with controls on a neuropsychiatric questionnaire.

8.2. Methods

8.2.1. The neuropsychiatric questionnaire was self-administered online via Qualtrics, and consisted of measures of depression, anxiety, OCD, impulsiveness, apathy, autism, ADHD, psychosis and personality. Participant recruitment, inclusion and exclusion criteria, and details of the neuropsychiatric assessment are detailed in chapter 6.

8.2.2. ALS relatives and controls were compared on their scores on these measures using Welch's t-tests (for parametric data) and Wilcoxon rank tests (for non-parametric data). Secondary analyses were carried to examine the effect of family history of ALS on neuropsychiatric traits. One-way ANOVAs (for parametric data) and Kruskal-Wallis tests (for non-parametric data) were carried out to compare the neuropsychiatric traits of FALS relatives, SALS relatives and controls. If a significant main effect was observed, post-hoc Bonferroni-Holm tests were carried out to compare sub-groups while controlling for multiple comparisons.

8.2.3. The effect of the C9orf72 repeat expansion on neuropsychiatric traits was examined by comparing ALS relatives who were C9orf72 positive gene carriers and ALS relatives who were C9orf72 negative. For this analysis, individuals with an intermediate C9orf72 status were considered as positive.

8.2.4. Significant differences between groups are illustrated using violin plots, and effect sizes are reported as Cohen's d, Pearson's r or omega squared. In cases where significant differences are observed between groups. Chi-square tests were carried out to examine if there are differences between groups in terms of clinically relevant scores (i.e., scores above clinical thresholds).

8.2.5. To control for the potentially confounding effect of age, multiple linear regressions were carried out. Each neuropsychiatric outcome was predicted using age and group status (i.e., ALS relative or control). This enabled us to examine the if ALS relatives had higher or lower scores, while holding age constant.

8.3. Results

8.3.1. Sample demographics

8.3.1.1. Of the total sample of 350 participants recruited in this study, 240 completed the online neuropsychiatric traits questionnaire. This consisted of 169 first- and second-degree relatives of ALS patients and 71 healthy controls. Controls and relatives were well matched in terms of sex and education. However, controls were younger than relatives. This is because most relatives were either siblings (largely in their 60's/70's) or offspring

(largely in their 20's/30's) of patients. In chapter 7, age specific norms were used to control for this difference in age. However, age specific normative data was not available for these neuropsychiatric questionnaires. Therefore, to control for the potentially confounding effect of age on neuropsychiatric traits, multiple linear regression models were carried out, including age as a predictor to control for its variance.

8.3.1.2. Of the 169 relatives, 115 were a relative of a patient with familial ALS, and 38 were asymptomatic C9orf72 gene carriers (see table 8.1.).

Twenty-eight unaffected relatives of ALS patients tested positive for the C9orf72 repeat expansion, 119 tested negative, and 10 had an intermediate C9orf72 repeat expansion (i.e., they had between 20 and 30 repeat expansions

Table 8.1. Demographic information of participants who completed the neuropsychiatric traits questionnaire.

	Control (n = 71)	Relative (n = 169)
Sex		
Female, n (%)	43 (60.6%)	104 (61.5%)
Male, n (%)	28 (39.4%)	65 (38.5%)
Age		
Age in years, mean (sd)	62.7 (11.5)	44.7 (16.2)
Education		
Years in education, mean (sd)	15.0 (3.48)	15.1 (3.81)
Family History		
FALS, n (%)		115 (68.0%)
SALS, n (%)		53 (31.4%)
C9orf72 repeat expansion status		
Negative, n (%)		119 (70.4%)
Intermediate, n (%)		10 (5.9%)
Positive, n (%)		28 (16.6%)

FALS – Familial ALS, SALS – Sporadic ALS

8.3.2. Depression

8.3.2.1. The presence and severity of depression were assessed using the Patient Health Questionnaire – 9 item version (PHQ-9). Histograms and Q-Q plots indicated that the assumption of normality was violated; therefore, a non-parametric Wilcoxon rank test was carried out to compare ALS relatives and healthy controls on PHQ-9 score. ALS relatives had significantly higher PHQ-9 score than controls, $W = 4514$, $p < .01$, $r = 0.17$ [95% CI: 0.03, 0.29] (see figure 8.1.).

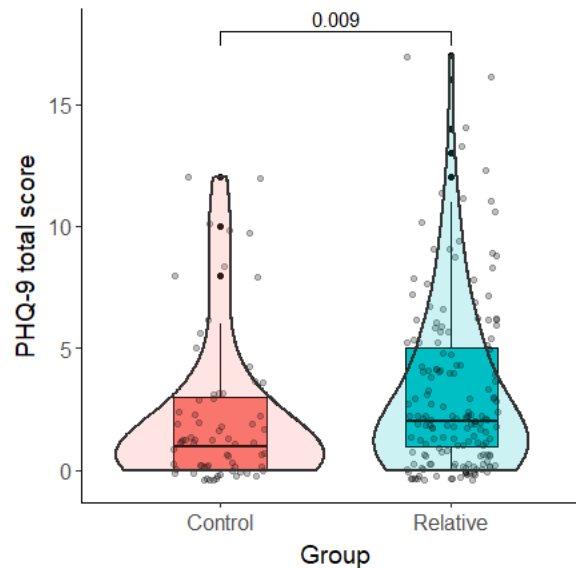


Figure 8.1. PHQ-9 scores in ALS relatives and healthy controls.

8.3.2.2. Due to the significant age difference between relatives and controls, and the potentially confounding effect this may have on PHQ-9 score, a multiple linear regression was carried out. PHQ-9 score was predicted using age and group status (i.e., control or ALS relative). A significant model was observed, $F(2, 231) = 8.68$, $p < .001$, $R^2 = .07$. Age was a significant

contributor to the model ($B = -.05$, $p < .001$). However, group status did not significantly add to the model, i.e., after controlling for age, relatives did not have significantly higher PHQ-9 score.

8.3.2.3. A chi-square test was carried out to examine if relatives had a higher frequency of participants above clinically relevant cut-offs (i.e., PHQ-9 score greater than or equal to 10). The chi-square test was not significant, indicating that relatives did not have a higher frequency of clinically relevant depression symptoms.

8.3.2.4. A Kruskal-Wallis test was carried out to examine the effect of family history of ALS on PHQ-9 scores, i.e., comparing relatives of FALS patients, relatives of SALS patients and healthy controls. A significant main effect was observed, $H(2) = 6.86$, $p < .05$. Post-hoc Wilcoxon rank tests with Bonferroni-Holm correction revealed that FALS relatives had significantly higher PHQ-9 scores than controls ($p < .05$; see figure 8.2.).

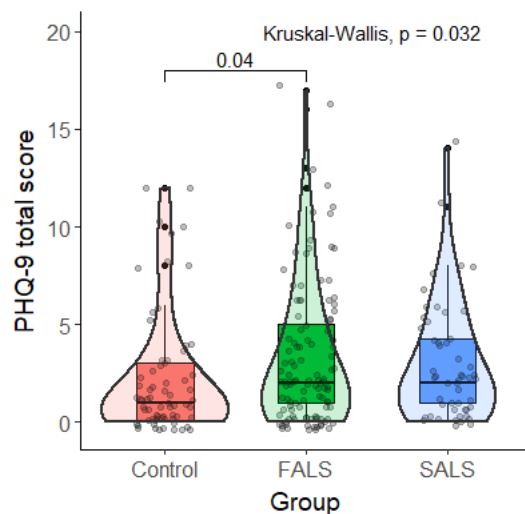


Figure 8.2. PHQ-9 scores in FALS relatives, SALS relatives and healthy controls.

8.3.2.5. Again, to control for the likely confounding effect of age, a multiple regression was carried out. PHQ-9 score was predicted using age and family history status (i.e., SALS, FALS or control). The model was a significant predictor of PHQ-9 score, $F(2, 230) = 5.77$, $p < .001$, $R^2 = .07$. Age was a significant contributor to the model ($B = -.05$, $p < .001$). However, family history was not, i.e., after controlling for age, family history was no longer associated with higher PHQ-9 score.

8.3.2.6. A chi-square test was carried out to compare FALS relatives, SALS relatives and controls on clinically relevant PHQ-9 scores (i.e., scores greater than or equal to 10). No significant differences were observed between the groups in their frequency of clinically relevant PHQ-9 scores.

8.3.2.7. A Wilcoxon rank test was carried out to compare C9orf72 positive relatives to C9orf72 negative relatives on PHQ-9 scores. No significant difference was observed.

8.3.2.8. Lastly, a multiple regression was carried out to examine the effect of C9orf72 status, controlling for age. A significant model was observed, $F(2, 151) = 5.75$, $p < .01$, accounting for 7% of variance. Both age ($B = -.05$, $p < .001$) and C9orf72 status ($B = -1.32$, $p < .05$) contributed significantly to the model, i.e., after controlling for age, C9orf72 status was associated with PHQ-9 status. Being positive for the C9orf72 repeat expansion was associated with a 1.32-unit lower PHQ-9 score.

8.3.2.9. In summary, there is no robust evidence of a difference between ALS relatives and controls in terms of depressive symptoms once age is

controlled for. Similarly, there is no robust evidence of an effect of family history on depression scores. Asymptomatic C9orf72 gene carriers had significantly lower depression scores than C9orf72 negative ALS relatives.

8.3.3. Anxiety

8.3.3.1. The presence and severity of anxiety were assessed using the Generalised Anxiety Disorder Assessment (GAD-7). Histograms and Q-Q plots indicated the assumption of normality was violated; therefore, a non-parametric Wilcoxon rank test was carried out to compare ALS relatives and healthy controls on GAD-7 score. ALS relatives had significantly higher GAD-7 score than controls, $W = 4315$, $p < .01$, $r = 0.2$ [95% CI: 0.07, 0.32] (see figure 8.3.).

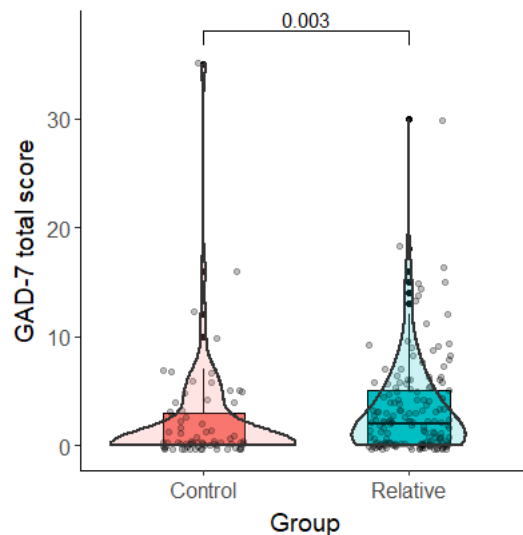


Figure 8.3. GAD-7 scores in ALS relatives and controls.

8.3.3.2. Due to the age difference between relatives and controls, and the potentially confounding effect this may have on GAD-7 score, a multiple linear regression was carried out. GAD-7 score was predicted using age and

group status (i.e., control or ALS relative). A significant model was observed, $F(2, 229) = 3.49$, $p < .05$, $R^2 = .03$. Age was a significant contributor to the model ($B = -.05$, $p < .05$). However, group status was not, i.e., after controlling for age, ALS relatives did not have significantly higher GAD-7 scores.

8.3.3.3. A chi-square test was carried out to examine if ALS relatives had a higher frequency of participants above clinically relevant cut-offs for GAD (i.e., GAD-7 score greater than or equal to 10). Chi-square was not significant, indicating that relatives did not have a higher frequency of clinically relevant anxiety symptoms.

8.3.3.4. A Kruskal-Wallis test was carried out to examine the effect of family history of ALS on GAD-7 scores, i.e., comparing relatives of FALS patients, relatives of SALS patients and healthy controls. A significant main effect was observed, $H(2) = 10.48$, $p < .01$. Post-hoc Wilcoxon rank tests revealed that FALS relatives had significantly higher GAD scores than controls ($p < .05$). No other post-hoc comparisons were significant (see figure 8.4.).

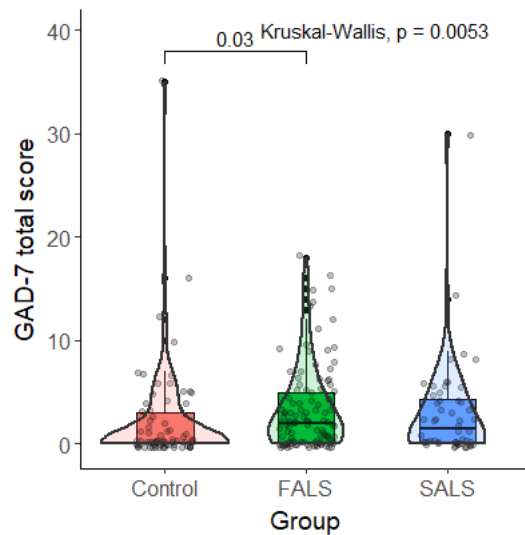


Figure 8.4. GAD-7 scores in FALS relatives, SALS relatives and controls.

8.3.3.5. To control for the possible confounding effect of age, a multiple regression was carried out. GAD-7 score was predicted using age and family history status (i.e., SALS, FALS or control). The model was a significant predictor of GAD-7 score, $F(2, 228) = 2.32$, $p < .05$, $R^2 = .03$. Age was a significant contributor to the model ($B = -.05$, $p < .001$). However, family history was not, i.e., after controlling for age, family history was no longer associated with higher GAD-7 score.

8.3.3.6. A chi square test was carried out to compare FALS relatives, SALS relatives and controls in terms of clinically relevant GAD-7 scores (i.e., scores greater than or equal to 10). No significant differences were observed between the groups in their frequency of clinically relevant GAD-7 score.

8.3.3.7. A Wilcoxon rank test was carried out to compare C9orf72 positive relatives to C9orf72 negative relatives on GAD-7 score. No significant difference was observed. A multiple regression was carried out to examine

the effect of C9orf72 status, controlling for age. The model was not significant, with neither age nor C9orf72 status significantly contributing to the model.

8.3.3.8. In summary, there is no robust evidence of a difference between ALS relatives and controls in terms of anxiety symptoms once age is controlled for. There is also no robust evidence of an effect of family history or C9orf72 status on anxiety scores.

8.3.4. OCD

8.3.4.1. The presence and severity of OCD symptoms were assessed using the Obsessive-Compulsive Inventory-Revised (OCI-R). Histograms and Q-Q plots indicated that the assumption of normality was violated; therefore, a non-parametric Wilcoxon rank test was carried out to compare ALS relatives and healthy controls on OCI-R score. No significant difference was found.

8.3.4.2. To control for age, a multiple linear regression was carried out. OCI-R score was predicted using age and group status (i.e., control or ALS relative). The model was not significant, with neither age nor groups status contributing significantly to the model.

8.3.4.3. A Kruskal-Wallis test was carried out to examine the effect of family history of ALS on OCI-R scores, i.e., comparing relatives of FALS patients, relatives of SALS patients and healthy controls. No significant main effect was observed. To control for the effect of age, a multiple linear regression was carried out. The model was not a significant predictor of

OCI-R score, with neither age nor family history contributing significantly to the model.

8.3.4.4. A Wilcoxon rank test was carried out to compare C9orf72 positive relatives to C9orf72 negative relatives on OCI-R scores. No significant difference was observed.

8.3.4.5. In summary, no significant differences were observed between ALS relatives and controls on OCD traits. There was no effect of family history, nor was there any effect for C9orf72 status.

8.3.5. Impulsiveness

8.3.5.1. Impulsiveness traits were assessed using the Barrett Impulsiveness Scale (BIS-11). Histograms and Q-Q plots indicated the assumption of normality was upheld; therefore, a Welch's t-test was carried out to compare ALS relatives and healthy controls on BIS-11 total score. No significant difference was found between ALS relatives and controls.

8.3.5.2. To control for age, a multiple linear regression was carried out. BIS-11 score was predicted using age and group status (i.e., control or ALS relative). The model was not significant, with neither age nor group status contributing significantly to the model.

8.3.5.3. Welch's independent samples t-tests were carried out to compare ALS relatives and controls on BIS-11 first and second order factors. No significant differences were observed on any BIS sub-score (see table 8.2.).

Table 8.2. BIS-11 sub-scores in ALS relatives and controls (n = 240).

BIS-11 sub-score	Control mean	ALS Relative mean	t	df	p
Attention	12.01	12.05	-0.09	139	0.93
Attention sub-score	7.19	7.47	-0.94	131	0.35
Cognitive instability	4.83	4.588	1.4	149	0.17
Motor	20.58	20.38	0.43	138	0.67
Motor sub-score	13.46	13.51	-0.11	146	0.91
Perseveration	7.12	6.87	0.99	116	0.33
Non-planning	22	23.07	-1.81	137	0.07
Self-control	11.26	11.78	-1.19	141	0.24
Cognitive complexity	10.74	11.29	-1.82	132	0.07

8.3.5.4. One-way ANOVAs were carried out to compare relatives of FALS patients, relatives of SALS patients and healthy controls on BIS-11 total and sub-scores. No significant main effects were observed. To control for the effect of age, a multiple linear regression was carried out. The model was not a significant predictor of BIS-11 total score, with neither age nor family history contributing significantly to the model.

8.3.5.5. Welch's independent samples t-test indicated that C9orf72 positive relatives had significantly lower BIS-11 total scores than C9orf72 negative relatives, $t(73.13) = 2.1$, $p < .05$, $d = 0.49$ [95%CI: 0.02, 0.95] (see figure 8.5.). A multiple linear regression was carried out to control for the effect of age. The model was not a significant predictor of BIS total score. However, C9orf72 status was a significant contributor to the model ($B = -3.52$, $p < .05$).

This indicates that C9orf72 positive relatives were associated with a 3.52-unit lower BIS-11 score, when controlling for age.

8.3.5.6. In summary, there is no evidence that ALS relatives are significantly more impulsive or over-controlled than controls. Similarly, no effect was observed for ALS family history. There is some evidence that asymptomatic C9orf72 positive relatives had significantly lower BIS-11 scores, indicating more over-controlled traits.

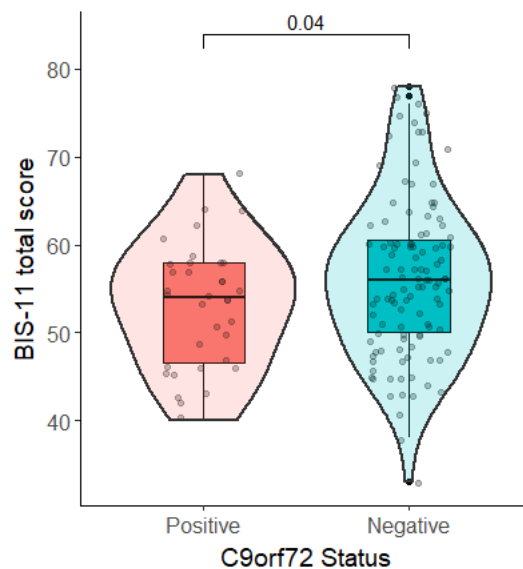


Figure 8.5. BIS-11 total score in C9orf72 positive and C9orf72 negative relatives.

8.3.6. Apathy

8.3.6.1. Apathy was assessed using the Dimensional Apathy Scale (DAS).

Histograms and Q-Q plots indicated the assumption of normality was upheld; therefore, Welch's t-tests were carried out to compare ALS relatives and healthy controls on DAS total, executive, emotional and initiation scores. No significant difference was found between ALS relatives and controls on DAS total, emotional or executive scores. However, ALS

relatives had significantly higher initiation apathy than controls, $t(133) = -2.72$, $p < .01$, $d = -0.47$ [95%CI: -0.82, -0.13] (see figure 8.6. below).

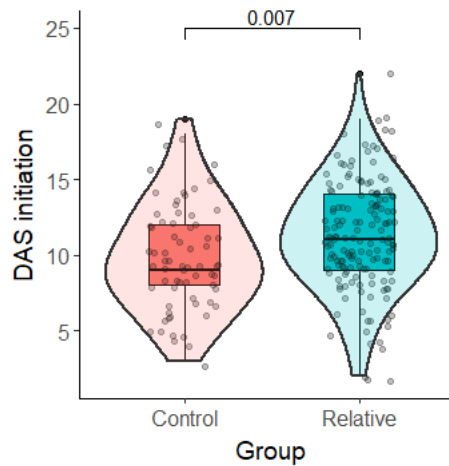


Figure 8.6. DAS initiation apathy in ALS relatives and controls.

8.3.6.2. To control for the effect of age on DAS initiation apathy, a multiple linear regression was carried out. Age and group status were entered as predictors. The model was significant, $F(2,222) = 4.34$, $p < .05$, accounting for 4% of variance. However, neither age, nor group status were significant contributors to the model, i.e., after controlling for age, relatives did not have significantly higher DAS initiation score.

8.3.6.3. A chi-square test was carried out to examine if relatives had a higher frequency of participants above clinically relevant cut-offs for apathy (i.e., DAS score greater than 38). Chi-square was significant, $\chi^2 = 4.20$, $p < .05$. ALS relatives were 1.89 times more likely than controls to have clinically relevant apathy, OR = 1.89 [95% CI: 3.5, 1.02].

8.3.6.4. One-way ANOVAs were carried out to compare relatives of FALS patients, relatives of SALS patients and healthy controls on DAS total,

executive, emotional and initiation scores. No significant main effect was observed for DAS total, executive or emotion scores. However, a significant main effect was observed for initiation apathy, $F(2,222) = 3.94$, $p < .05$. Post-hoc Bonferroni-Holm comparisons indicated that FALS relatives had significantly higher initiation apathy than controls ($p < .05$, see figure 8.7. below).

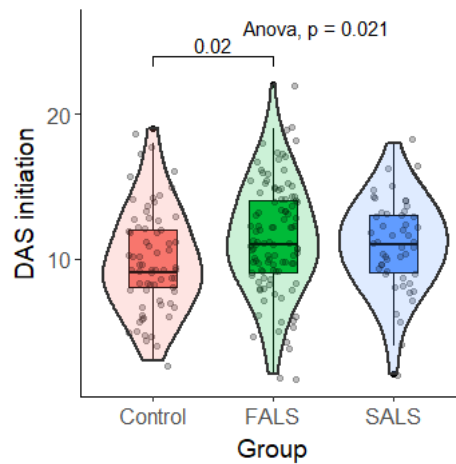


Figure 8.7. DAS initiation apathy in FALS relatives, SALS relatives and controls.

8.3.6.5. To control for the effect of age on DAS initiation scores, a multiple linear regression was carried out. Age and family history were entered as predictors. The model was significant, $F(3,221) = 3.03$, $p < .05$ accounting for 4% of variance. However, neither age, nor family history were significant contributors to the model, i.e., after controlling for age, FALS relatives did not have significantly higher DAS initiation apathy.

8.3.6.6. Welch's independent samples t-test found no significant difference between C9orf72 positive and C9orf72 negative relatives on any DAS total score. A multiple linear regression was carried out to control for the effect of

age. The model was not a significant predictor of DAS total score, with neither age nor C9orf72 status significantly contributing to the model.

8.3.6.7. Wilcoxon rank tests were carried out to compare C9orf72 positive and C9orf72 negative ALS relatives on DAS sub-scores. No significant differences were observed on DAS executive or initiation apathy. However, C9orf72 positive relatives had significantly higher emotional apathy than C9orf72 negative relatives, $W = 1417$, $p < .05$, $r = .2$ [95%CI: 0.03, 0.36]. A multiple linear regression was carried out to examine the effect of C9orf72 status on emotional apathy, controlling for age. The model was not a significant predictor of emotion apathy, $F(2,143) = 2.87$, $p > .05$. However, C9orf72 status was a significant contributor to the model ($B = 1.15$, $p < .05$), i.e., after controlling for age, C9orf72 positive individuals were associated with a 1.15-unit higher emotional apathy.

8.3.6.8. In summary, whether ALS relatives have significantly higher apathy once age is controlled for is unclear. The null finding of the regression model may be due to the reduced power that comes from including an extra predictor. The fact that age did not significantly contribute to the model suggests that age may not be a confounding factor and that the regression to control it was unnecessary. Ignoring the age discrepancy between groups, ALS relatives had higher apathy scores that were clinically relevant. There is also some evidence that ALS relatives with the C9orf72 repeat expansion have greater emotional apathy, even controlling for age.

8.3.7. Autism

8.3.7.1. Autism spectrum disorder (ASD) traits were assessed using the Autism Spectrum Quotient (AQ). Histograms and Q-Q plots indicated the assumption of normality was upheld; therefore, a Welch's t-test was carried out to compare ALS relatives and healthy controls on AQ total score. No significant difference was found between ALS relatives and controls. Only one participant scored above the clinically meaningful cut-off for autism traits.

8.3.7.2. Welch's independent samples t-tests were carried out to compare ALS relatives and controls on AQ subscales: social skills, communication, imagination, attention to detail and attention switching. No significant differences were observed between ALS relatives and controls on social skills, communication, imagination or attention switching. However, relatives had significantly higher attention to detail scores than controls, $t(122.21) = -2.88$, $p < .01$, $d = -0.52$ [95%CI: -0.88, -0.16] (see figure 8.8. below).

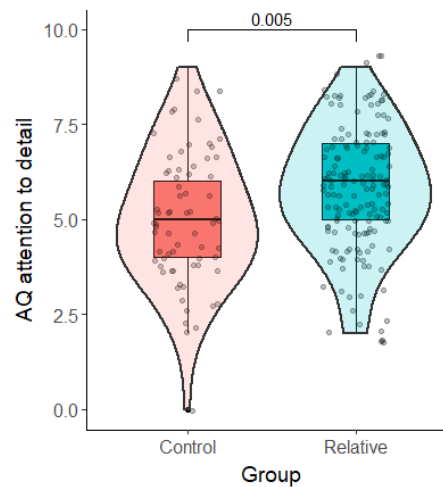


Figure 8.8. AQ attention to detail in ALS relatives and controls.

8.3.7.3. To control for the potentially confounding effect of age on AQ sub-scores, multiple linear regressions were carried out. Age and group status were entered into the model, predicting AQ attention to detail. A significant model was observed, $F(2,223) = 4.39$, $p < .05$, accounting for 4% of variance. Age was not a significant contributor to the model. Group status was a significant contributor ($B = 0.68$, $p < .05$), i.e., after controlling for age, ALS relatives still had significantly higher attention to detail scores.

8.3.7.4. A one-way ANOVAs were carried out to compare relatives of FALS patients, relatives of SALS patients and healthy controls on AQ total and sub-scores. No significant main effect was observed for AQ total, social skills, attention switching, communication or imagination scores. However, a significant main effect was observed for attention to detail, $F(2,223) = 5.55$, $p < .01$. Post hoc Bonferroni-Holm comparisons indicated that both FALS relatives and SALS relatives scored significantly higher than controls (see figure 8.9. below).

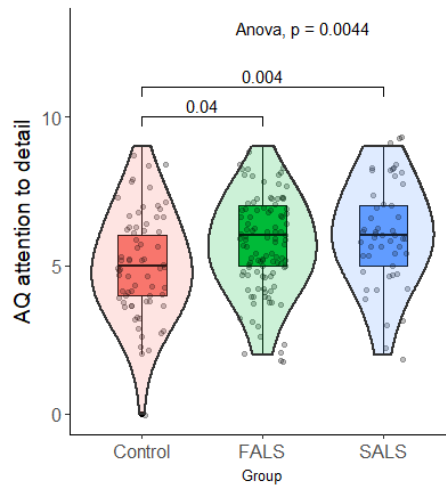


Figure 8.9. AQ attention to detail in FALS relatives, SALS relatives and controls.

8.3.7.5. A multiple linear regression was carried out to examine the effect of family history on attention to detail score, controlling for age. Age and family history were entered into the model, predicting AQ attention to detail scores. A significant model was observed, $F(3,222) = 3.75$, $p < .05$, accounting for 5% of variance. Age was not a significant contributor to the model. FALS family history was not a significant contributor to the model, but SALS history was, i.e., after controlling for age, SALS relatives had significantly higher attention to detail scores.

8.3.7.6. Welch's independent samples t-test found no significant difference between C9orf72 positive and C9orf72 negative relatives on AQ total score or sub-scores.

8.3.7.7. In summary, there is robust evidence that ALS relatives have significantly higher attention to detail traits than controls. This appears to be driven by relatives of SALS patients rather than relatives of FALS patients.

8.3.8. ADHD

8.3.8.1. The presence and severity of ADHD symptoms were assessed using the Adult ADHD Self-Report Scale – short form (ASRS). Histograms and Q-Q plots indicated the assumption of normality was violated; therefore, a non-parametric Wilcoxon rank test was carried out to compare ALS relatives and healthy controls on ASRS total score. No significant difference was found between ALS relatives and controls.

8.3.8.2. To control for age, a multiple linear regression was carried out. ASRS total score was predicted using age and group status (i.e., control or ALS relative). The model was not significant, with neither age nor group status contributing significantly to the model.

8.3.8.3. A Kruskal-Wallis test was carried out to compare relatives of FALS patients, relatives of SALS patients and healthy controls. No significant main effect was observed. To control for the effect of age, a multiple linear regression was carried out. The model was not a significant predictor of ASRS score, with neither age nor family history contributing significantly to the model.

8.3.8.4. Wilcoxon rank test found no significant difference between C9orf72 positive and C9orf72 negative relatives on ASRS total score. A multiple linear regression was carried out to control for the effect of age. The model was significant, $F(2,143) = 4.65$, $p < .05$, accounting for 6% of the variance in ASRS score. Both age ($B = -.02$, $p < .05$) and C9orf72 status ($B = -0.73$, $p < .05$) were significant contributors to the model, i.e., after controlling for

age, C9orf72 negative status was associated with a 0.73-unit higher ASRS total score (see figure 8.10.).

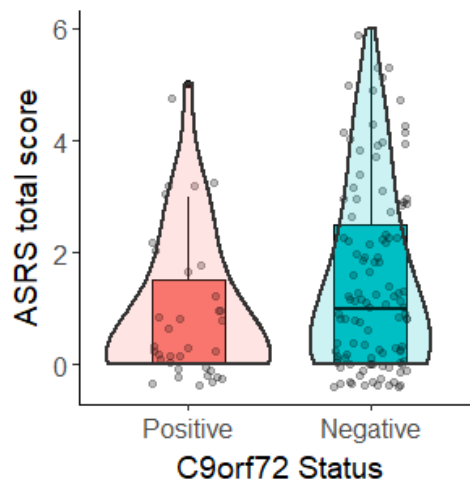


Figure 8.10. ASRS total score in C9orf72 positive and C9orf72 negative relatives.

8.3.8.5. In summary, there is no evidence of any difference in ADHD symptoms between ALS relatives and controls. There is some evidence that C9orf72 negative ALS relatives have significantly higher ADHD symptoms than C9orf72 positive relatives after controlling for age.

8.3.9. Psychosis

8.3.9.1. The positive symptoms of psychosis were assessed using the Community Assessment of Psychic Experience – Positive 15-item scale (CAPE-P15). Histograms and Q-Q plots indicated the assumption of normality was violated; therefore, non-parametric Wilcoxon rank tests were carried out to compare ALS relatives and healthy controls on CAPE total frequency and distress scores. ALS relatives had significantly higher CAPE-P15 frequency scores than healthy controls, $W = 4366$, $p < .01$, $r = 0.19$ [95%

CI: 0.07, 0.3] (see figure 8.11. below). No significant difference was observed on CAPE-P15 distress score.

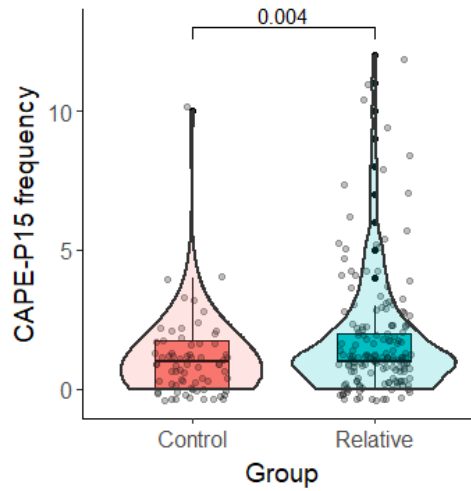


Figure 8.11. CAPE-P15 frequency scores in ALS relatives and controls.

8.3.9.2. To control for age, multiple linear regressions were carried out. CAPE-P15 frequency and distress scores were predicted using age and group status (control or ALS relative). The model was a significant predictor of CAPE-P15 frequency, $F(2,228) = 7.45$, $p < .001$, accounting for 6% of variance. Age was a significant contributor to the model ($B = -.02$, $p < .01$), however, group status did not significantly contribute to the model. The model was also a significant predictor of CAPE-P15 distress score, $F(2,228) = 4.5$, $p < .01$, accounting for 4% of variance. Again, age was a significant contributor to the model ($B = -0.02$, $p < .05$) but group status was not, i.e., after controlling for age, relatives did not have significantly higher CAPE-P15 frequency of distress scores than healthy controls.

8.3.9.3. Wilcoxon rank tests were carried out to compare ALS relatives and controls on CAPE-P15 sub-scores: persecutory ideation, bizarre experience

and perceptual abnormalities. ALS relatives had significantly higher persecutory ideation than controls, $W = 4446.5$, $p < .007$, $r = .18$ [95%CI 0.04, 0.29] (see figure 8.12.). No significant differences were observed in terms of bizarre experiences or perceptual abnormalities.

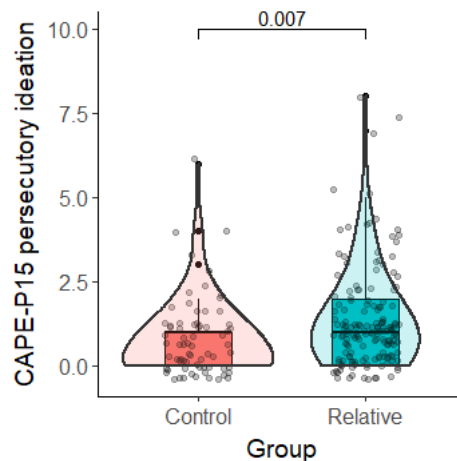


Figure 8.12. CAPE-P15 persecutory ideation in ALS relatives and controls.

8.3.9.4. To control for the effect of age on CAPE-P15 persecutory ideation, a multiple linear regression was carried out. Age and group status were entered into the model to predict CAPE-P15 persecutory ideation. A significant model was observed, $F(2,228) = 5.75$, $p < .01$, accounting for 5% of variance. Age was a significant contributor to the model ($B = -0.01$, $p < .05$). However, group status was not, i.e., after controlling for age, ALS relatives did not have significantly higher persecutory ideation.

8.3.9.5. A Kruskal-Wallis test was carried out to compare relatives of FALS patients, relatives of SALS patients and healthy controls. A significant main effect was observed for CAPE-P15 frequency score, $H(2) = 14.0$, $p < .001$.

Post hoc Wilcoxon rank tests found that FALS relatives scored significantly

higher than controls ($p < .05$) (see figure 8.13). A significant main effect was also observed on CAPE-P15 distress score, $H(2) = 10.45$, $p < .01$. However, post-hoc Wilcoxon rank tests found no significant differences between subgroups (see figure 8.13.).

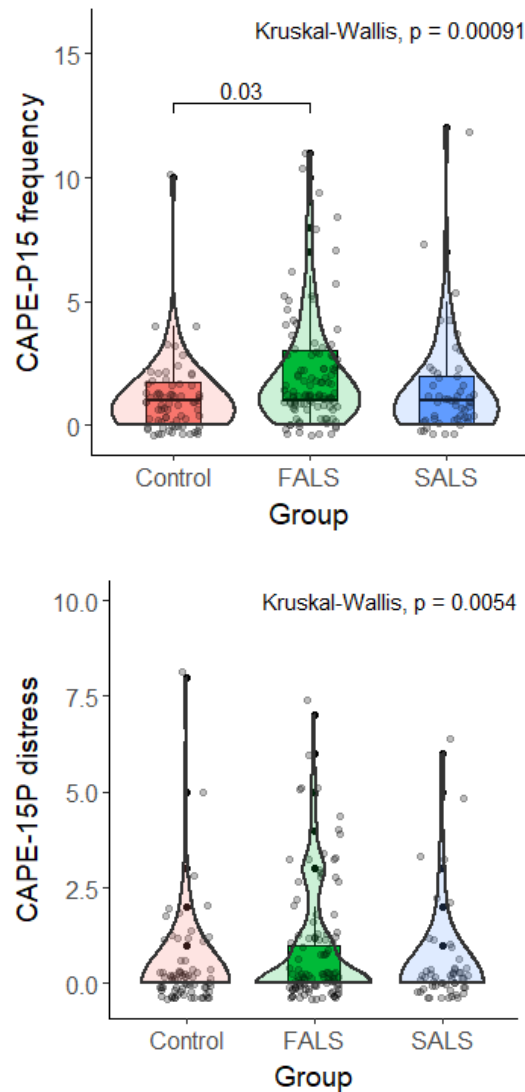


Figure 8.13. CAPE-P15 frequency and distress scores in FALS relatives, SALS relatives and controls.

8.3.9.6. To control for age, multiple linear regressions were carried out. CAPE-P15 frequency and distress scores were predicted using age and

family history status (i.e., FALS, SALS or control). The model was a significant predictor of CAPE-P15 frequency, $F(2,227)$ accounting for 7% of variance. Age was a significant contributor to the model ($B = -.02$, $p < .05$), however, group status did not significantly contribute to the model. The model was also a significant predictor of CAPE-P15 distress score, $F(3,227) = 4.02$, $p < .01$, accounting for 5% of variance. Again, age was a significant contributor to the model ($B = -.01$, $p < .05$), but group status was not, i.e., after controlling for age, FALS relatives did not have significantly higher CAPE-P15 frequency or distress scores than healthy controls.

8.3.9.7. Kruskal-Wallis tests were carried out to compare FALS relatives, SALS relatives and healthy controls on CAPE-P15 sub-scores. A significant main effect was observed for persecutory ideation, $H(2) = 15.54$, $p < .001$. Post hoc Wilcoxon rank tests indicated that FALS relatives scored significantly higher than both SALS relatives and controls (see figure 8.14.). No significant main effect was observed for the bizarre experiences or perceptual abnormalities sub-scores of the CAPE-P15.

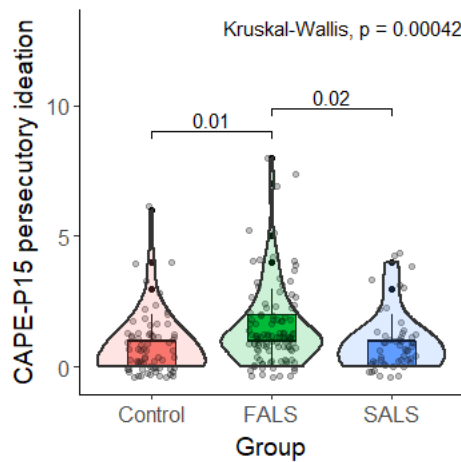


Figure 8.14. CAPE-P15 persecutory ideation in FALS relatives, SALS relatives and controls.

8.3.9.8. Wilcoxon rank test found no significant difference between C9orf72 positive and C9orf72 negative relatives on CAPE-P15 frequency or distress scores. Multiple linear regressions were carried out to control for the effect of age. The models were not significant predictors of CAPE-P15 frequency or distress scores, with neither age, nor C9orf72 status significantly contributing to the models.

8.3.9.9. Kruskal-Wallis tests found no significant differences between C9orf72 positive and C9orf72 negative relatives on CAPE-P15 sub-scores. Multiple linear models were constructed, examining the effect of family history (FALS, SALS and controls) on CAPE-P15 sub-scores. A significant model was observed for persecutory ideation, $F(3,227) = 5.86$, $p < .001$, accounting for 7% of variance. Age was a significant contributor to the model ($B = -0.01$, $p < .05$), but family history was not.

8.3.9.10. In summary, there is no robust evidence of a difference between ALS relatives and controls on CAPE-P15 frequency or distress total scores,

or sub-scores once age is controlled for. There was also no robust evidence of a difference between C9orf72 positive and negative ALS relatives.

8.3.10. Personality

8.3.10.1. Personality traits were measured using the Ten-Item Personality Inventory (TIPI). The TIPI consists of 10 questions, examining the big 5 personality traits: extroversion, agreeableness, conscientiousness, emotional stability and openness to experience. Welch's independent samples t-test found that relatives had significantly lower conscientiousness, $t(114.68) = 3.03$, $p < .01$, and openness to experience scores, $t(145.39) = 3.25$, $p < .01$ (see figure 8.15.).

8.3.10.2. To control for the effect of age, multiple linear regressions were carried out, including age and group status as predictors. The model was a significant predictor of conscientiousness score, $F(2,220) = 5.14$, accounting for 4% of variance. Age was not a significant contributor to the model, but group status was ($B = -.39$, $p < .01$). The model was also able to significantly predict openness score, $F(2,220) = 4.83$, $p < .01$, accounting for 4% of variance. Again, age was not a significant contributor to the model, but group status was ($B = -.47$, $p < .01$), i.e., after controlling for age, relatives still had significantly lower conscientiousness and openness to experience scores.

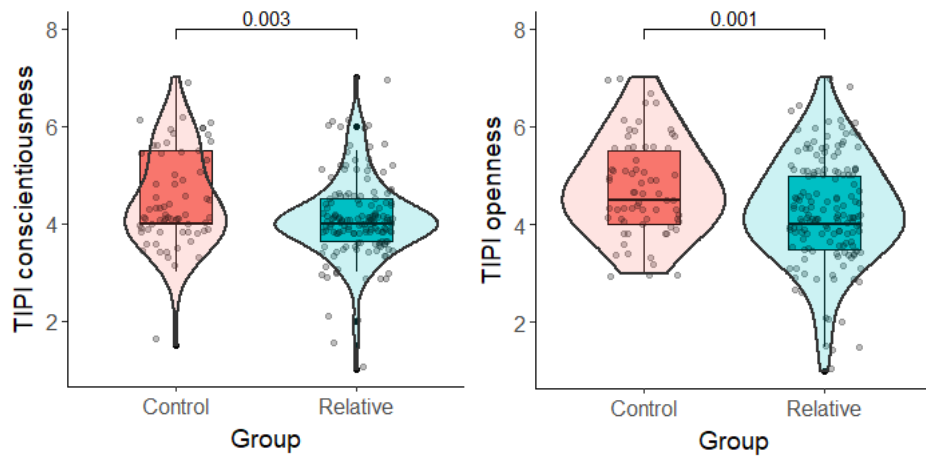


Figure 8.15. TIPI conscientiousness and openness to experience in ALS relatives and controls.

8.3.10.3. One-way ANOVAs were carried out to compare FALS relatives, SALS relatives and controls on TIPI personality scores. Significant main effects were observed for conscientiousness, $F(2,220) = 5.16$, $p < .01$, and openness to experience, $F(2,220) = 5.82$, $p < .01$. Post-hoc Bonferroni-Holm tests were carried out to compare FALS relatives, SALS relatives and controls on TIPI conscientiousness and openness traits. FALS relatives had significantly lower openness to experience scores than controls ($p < .01$). FALS relatives also had significantly lower conscientiousness scores than both controls ($p < .01$) and SALS relatives ($p < .05$) (see figure 8.16.).

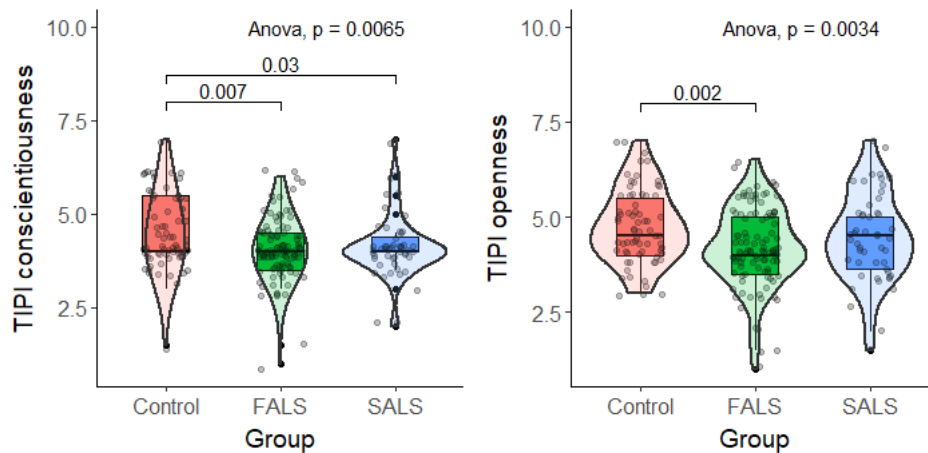


Figure 8.16. TIPI conscientiousness and openness to experience in FALS relatives, SALS relatives and controls.

8.3.10.4. To control for age, multiple linear regressions were carried out including age and family history as predictors. The model was a significant predictor of TIPI conscientiousness score, $F(3,219) = 3.43$, $p < .05$, accounting for 5% of variance. Age was not a significant contributor to the model ($p > .05$). Family history was a significant contributor to the model, with both FALS relatives ($B = -.4$, $p < .05$) and SALS ($B = -.36$, $p < .05$) associated with significantly lower conscientiousness scores than controls, i.e., after controlling for age, both FALS relatives and SALS relatives had significantly lower conscientiousness traits than controls.

8.3.10.5. The model was also a significant predictor of TIPI openness scores, $F(3, 219) = 3.88$, $p < .01$, accounting for 5% of variance. Again, age was not a significant contributor to the model ($p > .05$). Family history significantly contributed to the model, however only FALS relatives were associated with lower openness scores ($B = -.32$, $p < .01$) i.e., after controlling for age, FALS relatives had significantly lower openness traits than controls.

8.3.10.6. Welch's independent samples t-tests found no significant differences between C9orf72 positive and C9orf72 negative relatives on any TIPI personality traits. Multiple linear regressions were carried out to control for age. No models were significant predictors of TIPI scores.

8.3.10.7. In summary, there is robust evidence that ALS relatives have significantly lower conscientiousness and openness personality traits. These traits cluster more so in relatives of FALS patients than relatives of SALS patients. No differences were observed between C9orf72 positive and C9orf72 negative ALS relatives on any personality trait.

8.4. Summary of findings

8.4.1. Overall, few differences were found between ALS relatives and healthy controls on neuropsychiatric traits. Initial comparisons indicated that ALS relatives had higher anxiety, depression and psychosis traits, however, these findings were no longer significant after controlling for age. There were also no robust differences between relatives and controls in terms of OCD or impulsiveness traits.

8.4.2. ALS relatives had significantly lower conscientiousness and openness to experience traits than controls. This was driven largely by FALS relatives and was significant even when controlling for age.

8.4.3. C9orf72 negative individuals had significantly higher impulsivity and ADHD traits than C9orf72 positive individuals, even when controlling for age.

8.5. Discussion

8.5.1. This study is the first, in-depth characterization of the neuropsychiatric traits of unaffected ALS relatives. Unlike the analysis of neuropsychological tests, relatives showed few differences from controls. Consequently, these results are at variance with findings of previous aggregation studies, which purported a higher prevalence of schizophrenia/psychosis, suicide, OCD, alcoholism and autism in Irish ALS kindred (102,361).

8.5.2. The most reliable finding of these aggregation studies, (102,361) which was supported by genetic analysis (136), is that ALS relatives have a higher prevalence of schizophrenia/psychosis. Direct assessment of psychotic traits using the CAPE-P15 did not support this association once age was controlled for. This null finding may be due to the limitations of the CAPE-P15, which is typically used as a screening measure, and validated primarily in adolescent populations (341). Furthermore, the CAPE-P15 measures positive psychotic symptoms but does not address negative symptoms.

8.5.3. Another potential reason why there was no association with psychotic traits, is that these traits are likely present in a relatively small number of relatives, and this study did not have sufficient power to detect a small effect size. The aggregation studies mentioned have data on thousands of ALS relatives, from hundreds of kindred, enabling them to detect small, yet significant effects. For example, O'Brien et al. (135) had data 2116 ALS family members, with just 13 having a diagnosis of schizophrenia. While subclinical traits are expected to be more widespread than the clinical

diagnosis, this study likely had too few participants to detect an effect. This argument is made by McLaughlin et al., (136) who note that despite the genetic correlation between ALS and schizophrenia, large sample sizes are required to observe this epidemiologically.

8.5.4. Another potential factor that may explain the discrepancy between this study and previous aggregation studies is the method of data collection. In aggregation studies, history of psychosis was reported on a family history questionnaire and then verified using medical records. As a result, researchers got a relatively complete view of each ALS kindred. In contrast, this study recruited ALS relatives on a voluntary basis, which likely resulted in a self-selection bias. Anecdotally, when families were invited to participate, the research team were often instructed not to approach some members who were either ‘estranged’ or had ‘an unusual personality’. Social isolation and withdrawal from society is a core feature of schizophrenia (362). Thus, ALS relatives who may have displayed schizophrenia/psychosis traits were unlikely to participate.

8.5.5. One finding that was supported from aggregation studies, was that ALS relatives reported greater levels of ASD traits, which clustered in FALS relatives. However, this was specific to a sub-set of ASD traits centered around attention to detail. Attention to detail ASD traits relate to how individuals attend to fine grain details at the expense of more integrative perceptions (363).

8.5.6. Evidence that ALS relatives show higher levels of apathy than controls was mixed. ALS relatives had significantly higher apathy scores

than controls, however, this was no longer significant once age was controlled for. As age did not predict apathy score itself, this null finding may have been due to a reduction in power from adding a second predictor. Apathy in ALS relatives appeared to be specific to initiation apathy, i.e., difficulty generating thoughts or behaviours. This is the dimension of apathy that is most impaired in ALS patients (54), and is associated with abnormalities in anterior cingulate cortex (364).

8.5.7. Low scores of openness to experience and conscientiousness emerged as the most robust neuropsychiatric endophenotypes in this sample. Research into personality characteristics in ALS is limited, however some studies do suggest that ALS patients are more likely to display certain personality characteristics. A small mixed methods study of psychological functioning in ALS found that ALS patients exhibited a high degree of emotional control (365). This finding was supported by an American ALS cohort study which asked caregivers of ALS patients to rate the patients personality characteristics prior to their illness using the NEO-Personality Inventory (348). In this study patients with ALS were reported as having significantly lower openness to experience scores than patients with other chronic, progressive diseases, such as multiple sclerosis and cancer.

8.5.8. Individuals with low openness to experience typically attend to the problem at hand, suppressing emotional reactions. In the face of a terminal diagnosis, individuals with low openness to experience would tend to accept the clinicians view and focus on compliance with treatment. This may account for why ALS patients are anecdotally seen as ‘good patients’

(366,367). This personality characteristic and its tendency to promote problem-solving coping also coincide with the high scores on the attention to detail subscale of the Autism Quotient.

8.5.9. The results of this study compliment a recent analysis of neuropsychiatric disorders in Scottish ALS patients and their family members (137), where patients with a premorbid mood disorder had increased apathy post diagnosis. A family history of psychiatric disorders was associated with poorer visuospatial scores and a higher prevalence of behavioural change in patients. These finding were independent of C9orf72 status, implicating the involvement of unknown gene variants. Future research will be required to establish if the neuropsychiatric endophenotypes identified in this thesis are linked with cognitive and/or behavioural change in patients.

8.5.10. While ALS relatives appeared to have higher scores on apathy and autism traits, they did not have a higher prevalence of individuals above clinically relevant thresholds. This indicates that these neuropsychiatric traits are likely sub-clinical, and not easily observable without psychometric measurement.

8.6. Limitations

8.6.1. One of the key issues during analysis was to compare ALS relatives and controls, while addressing the difference in age between these groups. As normative data was unavailable, a regression-based approach was adopted to control for age. This appeared to be an effective approach for some neuropsychiatric measures, for example, age was associated with

depression, anxiety and psychosis scores. However, age did not appear to associate with OCD, impulsiveness, apathy, autism, OCD or personality score, and thus this approach may have been unnecessary.

8.6.2. While this study has primarily focused on genetic factors which may influence neuropsychiatric traits and disorders, it is equally important to consider the myriad psycho-social factors which may mediate and/or moderate these relationships. For example, individuals with schizophrenia disproportionately reside in areas with greater social deprivation and occupy lower socio-economic positions (368). While our sample of ALS relatives matched controls in terms of education, they may have scored lower on other socio-economic indicators, such as parental education and occupation. Future studies should implement more detailed assessment of socio-economic status to assess this issue.

8.6.3. Another potential confounder is the emotional impact that an ALS diagnosis may have had on family members, and how this influenced neuropsychiatric traits. Endophenotype studies often interpret differences between relatives and controls as indicative of genetic factors. However, the emotional trauma of having a loved-one receive a terminal diagnosis could certainly affect some of the outcome measures assessed. This could be especially true in families with a strong family history, where individuals have witnessed multiple relatives die from ALS. Furthermore, some participants may have been acting as the primary or secondary caregiver of a patient with ALS. The significant burden associated with caregiving may

have influenced scores on neuropsychiatric traits, particularly for measures of anxiety and depression.

8.6.4. Many of the neuropsychiatric questionnaires used in this study are short screening measures that may not have been sensitive enough to detect small group differences in neuropsychiatric traits. This was particularly the case for the measure of ADHD. The questionnaires used in this study were chosen to enable an exploratory analysis of neuropsychiatric traits in ALS relatives without overburdening participants with long assessments. However, future studies may benefit from applying more in-depth measurement of specific neuropsychiatric domains.

8.6.5. As mentioned in 8.5.4. this study is likely limited by selection bias. Relatives of ALS patients with abnormal neuropsychiatric traits may have been less likely to participate in a neuropsychological and neuropsychiatric research study. However, this is also likely to be true for healthy controls.

9. Chapter 9. Results 3: Differential Deficits and Clustering of Endophenotypes in Unaffected ALS Relatives

9.1. Introduction

9.1.1. The previous two chapters outline several potential endophenotypes of ALS. ALS relatives had significantly lower scores on intelligence, executive functioning, language and memory tasks, and significantly higher scores on autism attention to detail traits, and conscientiousness and openness personality traits than controls.

9.1.2. The purpose of this chapter was to examine these potential endophenotypes further. One of the key findings in Chapter 7 was that ALS relatives had significantly lower IQ than controls. However, IQ is strongly associated with other neuropsychological measures, such as executive functioning (369), memory (370) and language tasks (371), and may account for the deficits observed on these other functions. Thus, a series of analyses were carried out to parse the variance associated to IQ, i.e., the extent to which executive, memory and language deficits in ALS relatives are independent from IQ.

9.1.3. ALS is a considerably heterogenous disease, with different types of onsets, rates of progression, genetic contributors and cognitive and behavioural components. These varying profiles are likely due to different underlying processes, underpinned by differing genetic factors. Cluster analysis is a commonly used tool in ALS research to help differentiate

different sub-phenotypes of the condition, based on clinical, electrophysiological or cognitive data.

9.1.4. Clustering of clinical and demographic information suggests that 5 clusters of ALS groups exist, with varying survival outcomes (372). A recent cluster analysis of electrophysiological data suggest that ALS patients can be divided into 4 groups, reflecting varying degrees of disruption to somato-motor, frontotemporal and frontoparietal networks (373). Clustering of cognitive data in ALS suggests 4 patient subgroups: intact, mild impairment, moderate impairment and severe impairment (374).

9.1.5. Considering the numerous clusters observed in patients, it is possible that ALS relatives have a similar diversity of phenotypes. This may be due to known ALS risk genes such as C9orf72, or currently unknown risk genes. In this chapter, a multivariate cluster analysis was performed to examine if and how neuropsychological and neuropsychiatric outcomes relate to one another.

9.2. Methods

9.2.1. Participants

9.2.1.1. Participant recruitment, inclusion and exclusion criteria, the neuropsychological and neuropsychiatric assessments that were carried out, and the procedure that was followed are all detailed in chapter 6.

9.2.2. Statistical analysis

9.2.2.1. A series of hierarchical multiple linear regressions were carried out to determine if the cognitive deficits observed in ALS relatives were

independent of IQ. In each model, WASI-II FSIQ-2 was entered into the model first, followed by group status in step 2 (i.e., ALS relative vs control). This model was used to predict cognitive outcomes which were identified as potential endophenotypes in chapter 7. To reduce the number of highly correlated outcomes, just one cognitive outcome was chosen from each domain. These outcomes were the cognitive scores with the largest effect sizes, namely: FAS verbal fluency total score, CWIT inhibition errors, digit span backwards span, IGT trial 5 score, BNT phonemic score, RAVLT immediate recall, logical memory delayed recall and RCFT delayed recall.

9.2.2.2. K means clustering was carried out to examine whether and to what extent neuropsychological and neuropsychiatric endophenotypes cluster together. Cognitive tests with a high degree of missing data were excluded to maximize power. This included the SART and IGT, which some participants declined. Participants who did not have complete data on the remaining outcomes were excluded pairwise. This resulted in a reduced participant pool of 89 ALS relatives. The R package ‘NbClust’ was utilized to identify the ideal number of clusters in the neuropsychological and neuropsychiatric data. This package uses 15 different indices, such as the Hubert's gamma coefficient, the Dunn index and the corrected rand index, to determine the optimal number of clusters in a dataset. The optimal cluster number was identified and plotted. The characteristics of the different clusters was then explored.

9.3. Results

9.3.1. Role of IQ in cognitive endophenotypes

9.3.1.1. Verbal Fluency

9.3.1.1.1. A hierarchical linear regression was carried out to examine if ALS relatives had lower verbal fluency scores than controls, after controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.1.). Step 1 of the model was significant, with IQ accounting for 19% of the variance in verbal fluency. Step 2 of the model was also significant, adding an additional 3% of variance explained. This indicates that verbal fluency deficits in ALS relatives are not solely attributable to IQ. In real terms, ALS relatives had a 0.47-unit lower verbal fluency z-score than controls, holding IQ constant.

Table 9.1. Hierarchical multiple regression summary, predicting verbal fluency from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.19				<.001
Constant		-3.5	0.5		<.001
FSIQ-2		.03	0.005	0.44	<.001
Step 2	0.03				.005
Constant		-2.89	0.53		<.001
FSIQ-2		.03	0.005	0.40	<.001
Group		-0.47	0.17	-.18	.005

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = FAS verbal fluency z-score

9.3.1.2. CWIT Inhibition errors

9.3.1.2.1. A hierarchical linear regression was carried out to examine if ALS relatives made more CWIT inhibition errors than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.2.). Step 1 of the model was significant, with IQ accounting for 15% of the variance in CWIT errors. In this case, step 2 of the model was not significant, failing to explain any additional variance. This indicates that the CWIT inhibition deficits observed in ALS relatives are likely attributable to a difference from controls in IQ.

Table 9.2. Hierarchical multiple regression summary, predicting CWIT inhibition errors from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.15				<.001
Constant		-1.9	0.37		<.001
FSIQ-2		.02	0.004	0.39	<.001
Step 2	0.01				.15
Constant		-1.7	0.4		<.001
FSIQ-2		.02	0.004	0.37	<.001
Group		-0.18	0.12	-0.1	.15

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = CWIT inhibition errors z-score

9.3.1.3. Digit span backwards

9.3.1.3.1. A hierarchical linear regression was carried out to examine if ALS relatives had shorter digit span backwards scores than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.3.) Step 1 was significant, with IQ accounting for 16% of the variance in digit span performance. Step 2 in the model was also significant, adding an additional 4% of variance explained. This indicates that the digit span backwards deficit observed in ALS relatives is not entirely attributable to differences in IQ. In real terms, ALS relatives had a 0.43-unit lower digit span z-score than controls, holding IQ constant.

Table 9.3. Hierarchical multiple regression summary, predicting digit span backwards from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.16				<.001
Constant		-2.52	0.46		<.001
FSIQ-2		.03	0.005	0.40	<.001
Step 2	0.04				.004
Constant		-1.96	0.49		<.001
FSIQ-2		.03	0.004	0.37	<.001
Group		-0.43	0.15	-.19	.004

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = Digit span backwards z-score

9.3.1.4. IGT trial 5

9.3.1.4.1. A hierarchical linear regression was carried out to examine if ALS relatives had poorer IGT trial 5 performance than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.4.). In this case step 1 in the model was not significant, i.e., IQ did not predict IGT score. Step 2 in the model was significant, explaining 8% of the variance in IGT scores. This indicates that the IGT deficit observed in ALS relatives is completely independent from IQ. In real terms, ALS relatives had an 8.1-unit lower IGT t-score than controls, holding IQ constant.

Table 9.4. Hierarchical multiple regression summary, predicting IGT Trial 5 score from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.004				.53
Constant		40.7	8.74		<.001
FSIQ-2		0.05	0.08	0.06	.53
Step 2	0.08				.005
Constant		52.02	9.29		<.001
FSIQ-2		-0.01	0.08	-0.01	.91
Group		-8.1	2.8	-0.3	.005

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = IGT trial 5 t-score

9.3.1.5. BNT phonemic

9.3.1.5.1. A hierarchical linear regression was carried out to examine if ALS relatives had worse BNT phonemic scores than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.5.). Step 1 was significant, with IQ explaining 10% of variance in BNT score. Step 2 in the model was also significant, adding 6% of variance explained. This indicates that the BNT deficits observed in ALS relatives are not entirely attributable to differences in IQ. In real terms, ALS relatives had a 0.97-unit lower BNT phonemic z-score than controls, holding IQ constant.

Table 9.5. Hierarchical multiple regression summary, predicting BNT phonemic score from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.1				<.001
Constant		-5.22	0.83		<.001
FSIQ-2		0.04	0.008	0.32	<.001
Step 2	0.06				<.001
Constant		-3.98	0.87		<.001
FSIQ-2		0.03	0.008	0.27	<.001
Group		-0.97	0.26	-0.24	<.001

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = BNT phonemic z-score

9.3.1.6. RAVLT immediate recall

9.3.1.6.1. A hierarchical linear regression was carried out to examine if ALS relatives had poorer RAVLT immediate recall than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.6.). Step 1 in the model was significant, with IQ accounting for 23% of variance in RAVLT recall. However, step 2 in the model was not significant, failing to add any variance explained. This indicates that the RAVLT immediate recall deficits in ALS relatives are attributable deficits in IQ.

Table 9.6. Hierarchical multiple regression summary, predicting RAVLT immediate recall score from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.23				<.001
Constant		-3.46	0.54		<.001
FSIQ-2		0.04	0.005	0.48	<.001
Step 2	0.01				.06
Constant		-3.02	0.58		<.001
FSIQ-2		0.04	0.005	0.46	<.001
Group		-0.33	0.18	-0.12	.06

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = RAVLT 5 trial total immediate recall z-score

9.3.1.7. Logical Memory delayed recall

9.3.1.7.1. A hierarchical linear regression was carried out to examine if ALS relatives had poorer logical memory delayed recall than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.7.). Step 1 in the model was significant, with IQ accounting for 21% of the variance in logical memory scores. However, step 2 in the model was not significant, indicating that the logical memory delayed recall deficits observed in ALS relatives are attributable to deficits in IQ.

Table 9.7. Hierarchical multiple regression summary, predicting LM delayed recall score from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.21				<.001
Constant		0.9	1.44		.53
FSIQ-2		0.1	0.01	0.46	<.001
Step 2	0.03				.06
Constant		1.99	1.56		<.001
FSIQ-2		0.09	0.01	0.44	<.001
Group		-0.83	0.47	-0.12	.08

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = LM delayed recall scaled score

9.3.1.8. RCFT delayed recall

9.3.1.8.1. A hierarchical linear regression was carried out to examine if ALS relatives had poorer RCFT delayed recall than controls, controlling for IQ. WASI-II FSIQ-2 was entered into the model at step 1, and group status (i.e., ALS relative vs healthy control) was entered at step 2 (see model summary in table 9.8.). Step 1 in the model was significant, with IQ accounting for 7% of the variance in RCFT. However, step 2 in the model was not significant, indicating that the RCFT delayed recall deficit observed in ALS relatives is attributable to global differences in IQ.

Table 9.8. Hierarchical multiple regression summary, predicting RCFT delayed recall score from WASI-II FSIQ-2 and group status (n = 209).

	ΔR^2	B	SE B	β	p
Step 1	0.07				<.001
Constant		-4.02	1.08		<.001
FSIQ-2		0.4	0.01	0.27	<.001
Step 2	0.003				.52
Constant		-3.66	1.21		.003
FSIQ-2		0.04	0.01	0.26	<.001
Group		-0.24	0.37	-0.05	.52

FSIQ-2 = 2 Subtest Full Scale IQ

Dependent variable = RCFT delayed recall z-score

9.3.1. Cluster analysis

9.3.1.1. Participants

9.3.1.1.1. Eighty-nine ALS relatives were included in the cluster analysis.

The demographic and clinical details of this reduced cohort are outlined in table 9.9. below. While a large number of participants were excluded due to missing data, the cluster analysis sample has a similar mean age and education, and similar proportions in terms of gender, family history and c9orf72 status as the overall cohort (described in chapter 7).

Table 9.9. Demographic information of ALS relatives included in cluster analysis.

	ALS relative (n = 89)
Sex	
Female, n (%)	54 (61%)
Male, n (%)	35 (39%)
Age	
Age in years, mean (sd)	47.96 (16.2)
Education	
Years in education, mean (sd)	16.9 (3.36)
Family History	
FALS, n (%)	49 (55%)
SALS, n (%)	40 (45%)
C9orf72 repeat expansion status	
Negative, n (%)	74 (87%)
Positive or Intermediate, n (%)	11 (13%)

FALS – Familial ALS, SALS – Sporadic ALS

9.3.1.2. Number of clusters

9.3.1.2.1. K means cluster analysis of neuropsychological and neuropsychiatric data identified two distinct clusters of ALS relatives according to 15 different indices. A two-factor solution was also supported using the silhouette method (see figure 9.1.). The first cluster contained 57% of the sample ($n = 51$), and cluster 2 contained 43% ($n = 38$).

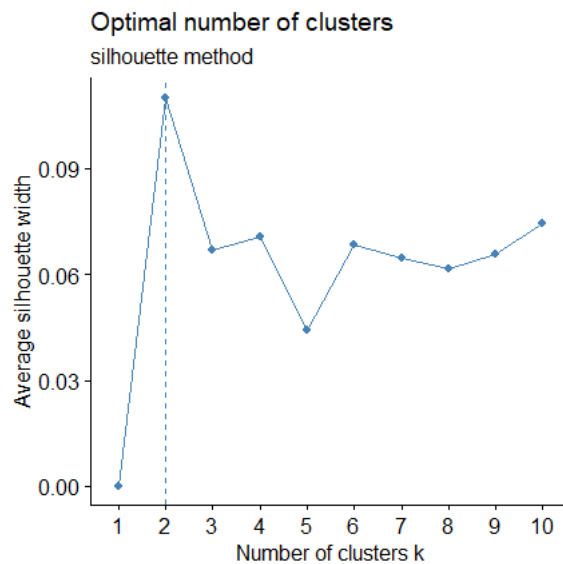


Figure 9.1. Silhouette graph of the optimal number of clusters.

9.3.1.2. The 2 clusters solution had a within cluster sum of variance of 10.4%. Cluster 1 had greater within cluster variance than cluster 2 (see figure 9.2.).

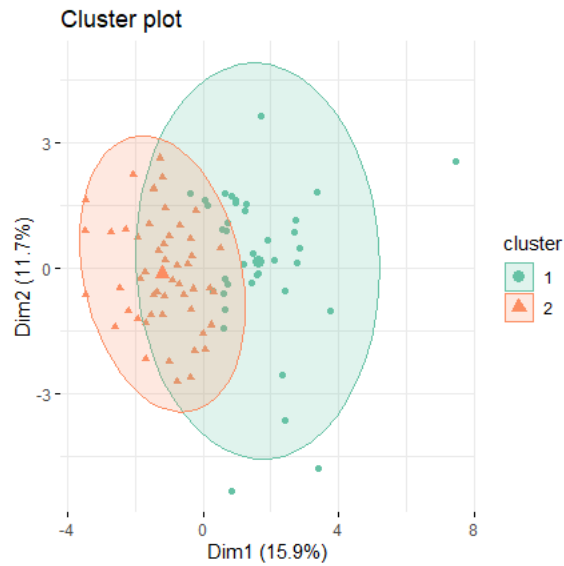


Figure 9.2. Cluster plot showing where participants lie from the cluster centroids.

9.3.1.2. Comparison of clusters

9.3.1.2.1. Clusters 1 and 2 were explored in terms of demographic and clinical characteristics. No significant differences were observed between the clusters in terms of C9orf72 status, family history of ALS (i.e., FALS vs SALS), age, education or handedness (see table 9.10.).

Table 9.10. Demographic and clinical characteristics of clusters 1 and 2.

	Cluster 1	Cluster 2	t/χ^2 (df)	p
	(n = 37)	(n = 48)		
Age, mean	47.16	48.55	-0.4 (78.96)	.69
Education, mean	16.44	17.25	-1.14 (81.54)	.26
Handedness, n left/n right	4/34	4/45	1.67 (1)	.43
Gender, n female/ n male	24/14	30/21	0.04 (1)	.85

C9orf72 status, n positive/ n negative	5/32	6/42	<.0001 (1)	.99

9.3.1.2.1. Clusters 1 and 2 were also compared in terms of the neuropsychological and neuropsychiatric profile of each cluster. Cluster 1 was characterized by significantly lower IQ, inhibition and social cognition scores than cluster 2. Cluster 1 was also associated with significantly higher anxiety, depression, psychosis, impulsiveness, extroversion, conscientiousness and emotional stability. In contrast, cluster 2 was associated with high IQ and higher inhibition, as well as higher apathy and autism attention to detail traits (see table 9.11. and figure 9.3.).

Table 9.11. Comparisons of clusters 1 and 2 on neuropsychological and neuropsychiatric scores.

	Cluster 1 (n = 37)	Cluster 2 (n = 48)	t(df)/W	p
WASI-II FSIQ-2	97.24	105	-3.02 (84.3)	.003
CWIT inhibition errors, scaled score	10.05	11.2	-2.2 (47.31)	.03
FAS verbal fluency, z-score	-0.36	-0.01	-1.59 (86.91)	.11
Digit span backwards, z-score	0.42	0.05	1.8 (76.63)	.08
RMET, z-score	-0.36	0.12	-2.28 (65.28)	.03
BNT phonemic, z-score	-2.24	-1.38	-1.86 (53.11)	.07
RAVLT immediate recall, z-score	0.25	0.63	-1.56 (80.14)	.12
RCFT delayed recall	-0.49	0.49	-1.81 (79.01)	.07
PHQ-9, raw score	3.87	1.94	1363	<.001
GAD-7, raw score	5.29	1.14	1630	<.001

CAPE-P15, raw score	2.58	.98	1398	<.001
OCIR, raw score	7.94	3.80	1424	<.001
AQ Attention to detail, raw score	5.08	6.27	-3.29 (67.44)	.002
DAS, raw score	36.16	43.02	-5.48 (68.85)	<.001
ASRS, raw score	2.05	0.67	1479	<.001
BIS, raw score	59.13	52.41	4.39 (70.7)	<.001
TIPI extroversion, raw score	4.87	3.94	4.96 (73.47)	<.001
TIPI agreeableness, raw score	4.83	4.49	1.65 (81.72)	.1
TIPI conscientiousness, raw score	4.26	3.93	2.01 (72.33)	.048
TIPI emotional stability, raw score	5.09	4.57	2.51 (84.14)	.01
TIPI openness, raw score	4.17	4.51	-1.39 (59.82)	.17

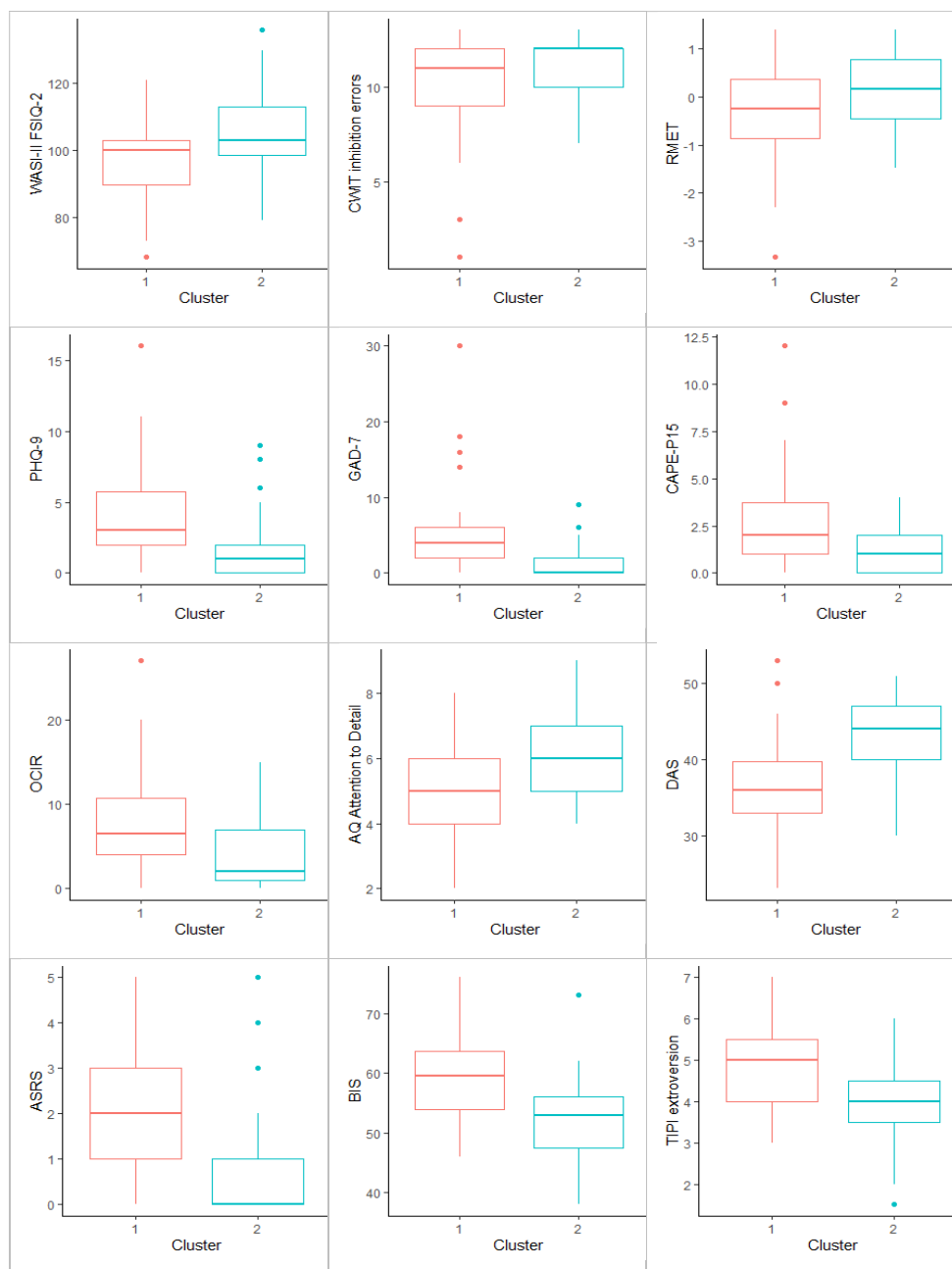


Figure 9.3. Comparisons between cluster 1 and cluster 2 on neuropsychological and neuropsychiatric measures.

9.4. Summary of results

9.4.1. Hierarchical regression analysis revealed that the memory and inhibition deficits observed in ALS relatives were attributable to deficits in IQ. Deficits in verbal fluency, working memory, and confrontational naming

were partially attributable to IQ, but relatives still had significantly lower scores even after holding IQ constant. IQ was not associated with IGT deficits, and therefore did not account for why ALS relatives performed poorly on this task.

9.4.2. Cluster analysis revealed two subgroups of ALS relatives. These subgroups were not driven by age, education, handedness, C9orf72 status or a stronger family history of ALS. Instead, these groups differed in their neuropsychological and neuropsychiatric profile. Cluster 1 was characterized by cognitive impairment and a range of neuropsychiatric traits. In contrast cluster 2 was characterized by normal cognitive functioning, and a higher degree of apathy and autism traits.

9.5. Discussion

9.5.1. These findings further elucidate the nature of the candidate endophenotypes identified in chapters 7 and 8. Regression analysis revealed that IQ accounted: entirely for deficits on inhibition and memory tasks, partially for deficits on verbal fluency, naming and working memory tasks, but not at all for emotion-based decision making. This pattern of results largely supports previous studies that parse out the contribution of IQ to neuropsychological tasks. Tasks with high cognitive complexity (i.e., multiple cognitive processes that rely on various white matter connections between frontal and posterior cortices) correlate highly with IQ, while focal tasks (i.e., more motor or sensory) do not (375) (376).

9.5.2. Studies on the association between IQ and inhibition report mixed results. Patient studies indicate that inhibition and IQ performance can be

differentially affected by frontal lobe damage, e.g., damage to the orbitofrontal cortex resulting in inhibition deficits does not result in concomitant IQ deficits (377). However, developmental studies show that inhibition is a strong predictor of age-related changes in IQ (378). Dempster (379) argues that inhibition is ‘the neglected dimension of intelligence’, as intelligence necessitates not only the ability to attend to goal relevant information, but also the ability to suppress goal irrelevant information.

9.5.3. Regression analysis also indicated that IQ accounted for deficits in verbal and non-verbal memory in ALS relatives. Studies of healthy aging indicate that intelligence (in particular fluid intelligence) is strongly correlated with memory performance (380). However, this association varies for the different processes of memory, such as encoding and rehearsal. IQ has a stronger influence on the early items of a memory task compared to later items (381).

9.5.4. Performance on the IGT, the measure of emotion-based decision making was not influenced by IQ. This supports previous findings that there is no association between IGT performance and IQ (382). There is also evidence that IGT performance is independent of executive functioning (383). These null associations support the validity of the IGT as a measure emotional-based decision making and the somatic marker. Impaired performance on this task indicates dysregulation of somatic markers (383) which is likely underpinned by dysfunction in the ventromedial prefrontal cortex and the amygdala (183).

9.5.5. For verbal fluency, digit span and confrontational naming, IQ partially explained deficits on these functions. Verbal fluency is typically viewed as a measure of language and executive functioning and is a sensitive marker of impairment in dementia (384). The results of this study support several previous studies that show a strong relationship between verbal fluency and IQ (385,386). The ability to generate words under a set of rules relies on both frontal and left temporal lobes. The fact that ALS relatives have significant deficits, even after IQ is held constant, suggests the presence of a more specific impairment, potentially underpinned by dysfunction of the supervisory attentional system and the dorsolateral prefrontal cortex (226).

9.5.6. This study's findings also add to the extensive evidence that IQ and working memory are closely associated, with meta-analysis estimating an association of $r = \sim 0.48$ (387). Intelligence appears to be most influential on the short-term memory aspect of working memory (388), while other components of working memory, such as mental speed, updating and attention are relatively independent of IQ.

9.5.7. Overall, the regression analysis suggests that IQ deficits are central to the neuropsychological endophenotype of ALS, contributing to inhibition, memory, naming, verbal fluency and working memory deficits in ALS relatives. While not within the scope of this research project, future studies could similarly parse out the variance explained by executive functioning in various non-executive tasks. This has already been applied in ALS patients for memory (389,390) and social cognition (360) tasks, and has helped to more accurately define the nature of the cognitive deficits observed in ALS.

9.5.8. Cluster analysis suggests the presence of two distinct subgroups of ALS relatives. One with a normal range of cognitive scores, high levels of apathy and high attention to detail autism traits; and another with cognitive deficits and wide range of neuropsychiatric traits. ALS relatives did not cluster into 4 groups, as has been reported in ALS patients (374), whereby individuals clustered based on the severity of cognitive impairment. These findings also diverge from the 4-factor solution observed based on EEG data in patients, where each group had distinct patterns of network dysfunction (373).

9.5.9. While the cluster solution of unaffected relatives is different to that observed in patients, it is strikingly similar to a study of unaffected relatives of schizophrenia patients (391), which also observed a 2-factor solution, 1 subgroup with cognitive impairments and 1 that was indistinguishable from controls. This indicates that unaffected relatives may be less heterogenous in their presentation than those with the clinical syndrome. Future studies could examine if these 2 subgroups indicate different levels of risk for ALS, FTD and neuropsychiatric illness. Based on their trait profile, cluster 2 likely reflects a group at elevated risk of Autism, while cluster 1 would likely be at higher risk for mood disorders, schizophrenia/psychosis and OCD.

9.6. Limitations

9.6.1. Cluster analysis was particularly impacted by missing data, with a significant portion of participants and cognitive tasks removed from the analysis. This likely limited the capacity of the analysis to uncover effects for family history and C9orf72 status. It is also likely that participants with

more missing data are likely those with more severe cognitive deficits or greater neuropsychiatric traits, resulting in a more biased cohort.

9.6.2. Due to covid-19 pandemic, and its' impact on control recruitment, cluster analysis could not be performed on healthy controls. Future studies should carry out a similar cluster analysis in controls to verify the extent to which the clusters observed in ALS relatives reflect distinct subgroups, and the extent to which these traits naturally cluster in the general population.

10. Chapter 10. Discussion, limitations and conclusions

10.1. Chapter outline

10.1.1. The primary objective of this thesis was to identify and explore potential neuropsychological and neuropsychiatric endophenotypes of ALS. To achieve this objective, 6 aims were specified. Aim 1 was to evaluate the equivalency and practice effects of ECAS versions A, B and C, to enable robust longitudinal measurement of cognition in ALS. Aim 2 was to study the effect of cognitive reserve on cognitive decline in ALS. Aim 3 was to compare the neuropsychological profile of ALS relatives and controls. Aim 4 was to compare the neuropsychiatric profile of ALS relatives and controls. Aim 5 was to examine the effect of IQ on cognitive endophenotypes in ALS. Aim 6 was to examine if neuropsychological and neuropsychiatric endophenotypes clustered into distinct subgroups in ALS relatives.

10.1.2. The current chapter highlights the key findings of this thesis in relation to these aims, and in relation to the literature. The limitations of the current study are outlined in detail, with suggestions for future studies that may stem from this project.

10.2. Robust measurement of cognition over time

10.2.1. In order to facilitate the robust measurement of cognitive change over time, the equivalency and potential practice effects of ECAS versions A, B and C were examined. Overall, alternative ECAS versions were highly comparable but not strictly equivalent. ECAS A was equivalent to ECAS B and C, however, ECAS B and C were not equivalent.

10.2.2. Serial administration of ECAS A-B-C produced small practice effects, with significant improvements from time 1 to time 2, but no further improvement at time 3. The practice effects observed from administering alternative ECAS versions (i.e., ECAS A-B-C) were far less than those observed from repeating ECAS version A (i.e., ECAS A-A-A) (262).

10.2.3. These findings suggest that researchers should be cautious when comparing scores on different versions of the ECAS and when interpreting cognitive change over time. To facilitate the interpretation of cognitive change over time, reliable change index (RCI) scores were developed. These take into consideration practice effects and provide thresholds for clinically meaningful cognitive decline in patients. This is particularly relevant for individuals with high baseline cognition, whose cognitive decline can often be missed using traditional cut-offs for impairment (i.e., 2 SD below a control mean). The results of this study facilitated the accurate assessment of cognition over time required to examine cognitive reserve in ALS.

10.3. The impact of cognitive reserve on ALS

10.3.1. Longitudinal analysis of cognition in ALS revealed a strong association between cognitive reserve (CR) and preserved executive, language and social cognitive functioning in ALS patients. Early in their disease, individuals with higher CR had better performance on ECAS, social cognition, verbal fluency, inhibition and confrontational naming. Over time, individuals with high CR maintained this higher performance compared to lower reserve individuals.

10.3.2. The role of CR on memory performance was less clear, suggesting that CR may have differential effects for different cognitive functions. Higher CR was associated with better baseline memory performance; however, memory scores declined quicker in the high CR group. This pattern of change over time has previously been observed in Alzheimer's disease (285). High CR individuals with Alzheimer's disease maintain normal functioning for longer, despite higher levels of pathology than those with lower CR. However, once the pathology reaches a certain threshold (enough to overcome the protective effect of CR), the advanced pathology in high CR individuals results in a steeper cognitive decline.

10.3.3. The findings of chapter 5 correlate well with other studies of CR in ALS that have subsequently been published. A cross-sectional study of an Italian ALS cohort found that patients with higher levels of CR had significantly higher scores on executive, verbal fluency and memory domains of the ECAS compared to patients with lower CR (392). Interestingly, higher CR was also associated with better motor functioning (measured by ALSFRS-R) in bulbar onset ALS patients, indicating that CR could potentially have protective effects on non-cognitive functioning. The major limitation of this study was that it did not measure cognitive change longitudinally and only used the ECAS, rather than a detailed neuropsychological battery, to measure cognition.

10.3.4. A cross-sectional study of CR in ALS explored the potential neuroimaging correlates of CR using ^{18}F -FDG-PET (288). This study found that higher CR was negatively correlated with brain metabolism in the right

anterior cingulate and bilateral medial frontal cortex. A negative correlation between medial frontal regions and the cerebellum found only in ALS patients suggested that the cerebellum may play a compensatory role in ALS (i.e., underpin the compensatory mechanism of CR in ALS). This supports recent neuroimaging findings that cerebellar and cerebro-cerebellar changes modulate, exacerbate or partially drive motor and non-motor symptoms of ALS (393). However, the major limitation of this study was that it only used education as a proxy of CR (rather than a multiple variable proxy), and only the ECAS was used to measure of cognition.

10.3.5. Another cross-sectional neuroimaging study, in a German ALS cohort, examined the neurological correlates of cognitive reserve in ALS (394). This study measured regional brain volume using MRI and assessed cognition using a full neuropsychological assessment. This study used a regression approach, with regional brain volume entered as a predictor, CR as a moderator and cognitive performance as outcomes. In this study, higher CR was associated with better performance on verbal fluency, working memory, verbal learning and recognition, and visuo-constructive ability.

10.3.6. The growing body of evidence on CR in ALS represents a promising modifiable factor that can be targeted by clinicians. To keep well cognitively, ALS patients could be encouraged to keep cognitively, physically and socially active. Modifiable risk factors for dementia may also be targeted to reduce the likelihood of impairment in ALS, such as hypertension, hearing impairment, smoking, obesity, depression, diabetes, alcohol consumption, and air pollution (395).

10.3.7. These risk factors can also be targeted at a governmental and societal level, as detailed by the Dementia prevention, intervention, and care: 2020 report by the Lancet Commission (395). All children should be provided with primary and secondary education. Hearing aids should be encouraged to reduce the risk from hearing loss. Healthy diet and exercise should be promoted to reduce risk from hypertension, obesity and diabetes. Air pollution should be reduced and smoking, and alcohol use discouraged.

10.4. Candidate cognitive endophenotypes for ALS

10.4.1. Comparisons between relatives of ALS patients and healthy controls revealed numerous candidate cognitive endophenotypes. ALS relatives scored significantly worse than controls across numerous cognitive domains. The largest effect sizes were observed for verbal fluency and confrontational naming, and moderate effect sizes were observed for inhibition, working memory, IQ, emotion-based decision making and memory.

10.4.2. The cognitive deficits observed in ALS relatives closely resemble those of ALS patients, i.e., deficits in executive and language domains, with verbal fluency particularly sensitive. In patients, these impairments are considered to reflect disruption to the supervisory attentional system (SAS) and the fronto-striatal circuits that underpin it (226). The SAS is a key component of Norman and Shallice's (396) model of executive functioning, responsible for monitoring deliberate action planning where novel tasks cannot be completed using existing schema, and for inhibiting habitual responses. Verbal fluency places heavy demands on executive processes, as

individuals are required to initiate effective retrieval strategies and to continuous switch between different retrieval strategies (397).

10.4.3. Concurrent verbal fluency deficits in ALS relatives may be due to a similar, more subtle disruption to the SAS and its underlying networks.

While neuroimaging or electrophysiological data were not collected on these participants, their cognitive deficits indicate abnormalities in the dorsolateral prefrontal circuit in those with verbal fluency impairment, and abnormalities in the orbitofrontal circuit in those with deficits in emotion-based decision making. These findings also support the model of ALS as a network disorder, starting in the motor cortex, cranial nerve motor nuclei and the spinal cord motor neurons and then spreading to prefrontal, ventral and medial frontal cortices, as well as parietal, temporal and deep grey matter structures. Future electrophysiological studies of ALS relatives, using event related potential (ERP) protocols, could further validate this model of pathophysiology in ALS.

10.4.4. The wide range of cognitive domains that appeared to be affected in ALS relatives raised the possibility that many of these deficits were secondary to deficits in IQ. A similar issue emerged in schizophrenia endophenotype research, where deficits in executive functioning, working memory, attention, inhibition, episodic memory and social cognition were all put forward as potential endophenotypes, leading some to suggest that the endophenotype of schizophrenia was simply a lower IQ (166).

10.4.5. In chapter 9, this issue was addressed by running a differential deficit analysis. This follows a similar approach implemented by Crawford et al.

(398), who used regression analysis to test if decline in executive functioning mediated age-related memory decline over and above decline in general cognitive ability. Differential deficit analysis suggested that inhibition and memory deficits in ALS relatives were entirely attributable to IQ differences. Deficits in verbal fluency, working memory and confrontational naming were only partially attributable to IQ.

10.4.6. These findings suggest that IQ deficits are a central component of the cognitive endophenotypes identified. However, the fact that ALS relatives had verbal fluency, naming and working memory deficits, even after controlling for IQ, suggests the presence of more specific impairments. Future research will be needed to further delineate how these putative endophenotypes relate to one another. In schizophrenia, it took years of investigation to uncover how different cognitive endophenotypes related to one another, and how they related to electrophysiological endophenotypes. By combining the most promising cognitive and electrophysiological endophenotypes, genetic studies were able to identify a number of novel genes (related to neuregulin and glutamate pathways), that gave new insight into the schizophrenia pathological mechanisms. (164). Endophenotype research in ALS will require a similar depth of research before their value in genetic studies is realized.

10.4.7. IGT deficits were completely independent of IQ, and indicate that ALS relatives may have dysfunctional somatic markers, i.e., an inability to generate normal emotional-based biasing body signals (383). This became more apparent as the task progressed from stage 1 to 5. A small cohort study

of ALS patients found a similar behavioural pattern of results, with worsening performance as the task progresses (399). This pattern in ALS patients and unaffected relatives likely reflects an inability to learn from win-lose contingencies, rather than a proclivity for risk making decisions (400). Such a mechanism may account for the higher prevalence of alcoholism and suicide in ALS kindred (401). A study of first-degree relatives of suicide completers found deficits on the IGT, particularly for the latter stages of the task (402). IGT performance may be a useful endophenotype of both ALS and suicidal behaviour, accounting for the observed link between them in aggregation studies. Somatic marker impairments are usually associated with ventromedial prefrontal cortex abnormalities, however, there is some evidence that IGT is reliant on dorsolateral prefrontal cortex activity (403), which is known to be affected in ALS.

10.4.8. While not examined in this thesis, cognitive reserve may play an important role in the cognitive endophenotypes of ALS relatives. A study of pre-manifest and prodromal Huntington's disease gene carriers found that higher CR was associated with slower cognitive decline and a slower rate of volume loss in the caudate and putamen (50). Furthermore, the strength of this association was stronger as an individual drew closer towards phenocconversion (i.e., going from asymptomatic to symptomatic). Future studies of unaffected ALS relatives should explore the potentially moderating effect that CR may play. If CR does play a role, unaffected relatives of ALS patients (particularly those with known risk genes) may

benefit from targeted interventions that aim to promote CR, and in doing so delay or slow down cognitive impairment and dementia.

10.4.9. Further neuroimaging and electrophysiology research will be needed to map the neurological correlates of the cognitive deficits observed in ALS relatives. Some studies have been carried out on asymptomatic C9orf72 positive relatives, identifying blood flow changes in the orbitofrontal, anterior cingulate and inferior parietal cortices, and the left middle frontal gyrus (148). However, these studies have ignored neuroimaging and electrophysiological markers in non-C9orf72 positive ALS relatives. The evidence from this thesis indicates that the non-C9orf72 ALS relative population is equally as affected in their neuropsychological and neuropsychiatric profile as those with C9orf72 and warrant equal attention.

10.4.10. While neuroimaging and electrophysiology studies of unaffected relatives are an important next step to take in understanding endophenotypes of ALS, it is possible that these endophenotypes may be more challenging to identify than cognitive or psychiatric traits. In bipolar disorder, where endophenotype research is well established, a meta-analysis of 77 studies found that unaffected relatives have deficits on verbal memory, sustained attention and executive functioning when compared to healthy controls (404). However, in contrast, neuroimaging studies have reported conflicting findings. Despite using similar methodologies and samples (unaffected relatives of bipolar patients), 5 studies found reduced grey matter volume in the insula and cerebellum, while 4 studies found increased grey matter volume insula and cerebellum (404). These conflicting findings may be due

to inadequate sample size, a common issue in neuroimaging studies, and publication bias. It is important that, as neuroimaging and electrophysiology studies of ALS relatives are undertaken, these pitfalls are avoided.

10.5. Candidate neuropsychiatric endophenotypes of ALS

10.5.1. Exploration of candidate neuropsychiatric endophenotypes was complicated by the lack of age-matched normative data. To control for the age differences between ALS relatives and controls, multiple linear regressions were carried out. From this analysis, 4 candidate neuropsychiatric endophenotypes were identified: greater initiation apathy, high scores on attention to detail autism traits, and low conscientiousness and openness to experience personality traits.

10.5.2. Previous aggregation (102,135) and genetic (136) studies show robust evidence that ALS relatives have a higher risk of psychotic disorders. In this sample, there was no evidence that ALS relatives, as a whole, had higher levels of psychotic traits once age was controlled for. However, when running cluster analysis, it emerged that a subgroup of ALS relatives did in fact have significantly higher schizophrenia/psychosis traits along with a range of other neuropsychiatric traits. These findings suggest that the epidemiological and genetic link between ALS and schizophrenia may be limited to a sub-group of ALS relatives. Future GWAS could utilize the CAPE-P15 as an outcome measure (along with clinical diagnosis) to identify novel risk genes associated with both ALS and schizophrenia.

10.5.3. Aggregation studies also highlighted a possible link between ALS and Autism Spectrum Disorder (ASD) (5), particularly for kindred with the

C9orf72 repeat expansion (138). In this study, ALS relatives did not have high Autism Quotient total scores (a measure of overall ASD traits), however they did have higher scores on the attention to detail sub-score, with and without controlling for age, suggesting the association between ALS and autism, may be more specific to this ASD trait.

10.5.4. Autism traits have not previously been examined in relation to ALS, likely due to the large discrepancy in age of onset of the 2 conditions.

However, the behavioural changes often observed in ALS share similar features to autism, such as impaired social cognition, communication deficits and increased behavioural rigidity. These impairments are potentially underpinned by impaired theory of mind (i.e., the capacity to recognize mental states in others) in both conditions (274,321). Evidence of neuroimaging and neuropsychological changes in pre-symptomatic C9orf72 carriers decades before symptom onset, raises the possibility that some forms of ALS are neurodevelopmental (405), similar to ASD.

10.5.4. Low openness to experience traits emerged as the most promising neuropsychiatric endophenotype identified by this thesis. It is highly heritable, with a twin study estimating heritability to be ~61% (406). Like all the big five personality traits, it is state independent and stable over time. Most crucially however, it appears to be present in both ALS patients and their unaffected relatives. It is possible that genes that predispose an individual to ALS may also predispose an individual to a more emotionally controlled and less open to experience personality. If this is the case, future

GWA studies could discover new ALS risk genes by including this personality trait as in their analyses.

10.6. Clustering of endophenotypes

10.6.1. K-means cluster suggested 2 distinct subgroups of ALS relatives.

Cluster 1 was characterized by cognitive impairment and higher neuropsychiatric traits. In contrast, cluster 2 was characterized by normal cognitive functioning, a higher degree of apathy and greater autism attention to detail traits. These clusters were not driven by demographic or clinical factors such as age, gender, education, family history or C9orf72 status, raising the possibility that they were driven by unknown gene variants.

10.6.2. The 2-factor solution observed in ALS relatives closely resembles a similar study of relatives of schizophrenia patients, where relatives either had cognitive deficits or not (391). It is unsurprising that unaffected relatives are less heterogenous in their presentation than those with the clinical syndrome, where there is much larger variation in level of disease progression and consequently more varying cognitive and EEG presentations.

10.6.3. These 2 clusters may indicate the presence of diverging risk of neuropsychiatric disease (and possibly varying risk of ALS phenotypes) in ALS kindred. This is supported by a recent study of Scottish ALS patients and their family members, where psychiatric illness in unaffected relatives was associated specifically with visuospatial decline and behavioural change in patients (137). This association may be associated with participants in cluster 1, who show a higher risk for mood disorders,

schizophrenia/psychosis and OCD. In contrast, cluster 2 may associate with ALS patients with normal cognitive functioning but a higher risk of initiation apathy.

10.6.4. Future research may explore the extent to which these 2 clusters in ALS relatives relate to behavioural clustering in patients. In ALS patients, behavioural change clusters into 5 distinct clusters, representative of differential network involvement. Unaffected ALS relatives in cluster 1 may associated with cognitive rigidity, reflecting alterations in the dorsolateral prefrontal cortex and temporo-limbic networks. Similarly, unaffected relatives with IGT deficits may be associated specifically with disinhibition and impaired impulse control, reflecting dysfunctional orbitofrontal, anterior insula and anterior cingulate pathways.

10.7. The effect of family history

10.7.1. Family history of ALS significantly affected the magnitude of the most robust endophenotypes identified. Relatives of FALS patients had poorer verbal fluency and lower scores on openness to experience and conscientiousness personality traits than both SALS relatives and controls.

10.7.2. The clustering of initiation apathy and verbal fluency deficits in FALS relatives might reflect disruption of the energization component of Stuss's model of executive functioning. Energization is defined by the ability to initiate and sustain responses (407). Under the framework of the supervisory attentional system, when external stimuli or motivation is absent, energization is required to activate low level perceptual or motor schemata. Impaired energization often manifests on neuropsychological

assessments as decreased word output on verbal fluency tasks and increased apathy scores. Energization deficits would also be expected to manifest on the SART; however, the lack of age specific normative data likely accounts for this null finding.

10.7.3. The cognitive profile of ALS patients and their unaffected relatives follows a very similar pattern to that observed in schizophrenia. A highly powered family-based study of Chinese schizophrenia patients and their parents found that both patients and their unaffected parents had deficits in verbal fluency, even after controlling for age, education and gender (347). The effect size was larger for patients and parents with a family history of schizophrenia compared to sporadic cases. This illustrates the likely pattern of effect sizes expected in endophenotype studies of ALS, i.e., strongest effects for familial cases due to an increased genetic load. It also indicates that verbal fluency deficits are unlikely to be specific to just ALS relatives, but rather an endophenotype of both ALS and neuropsychiatric disease.

10.8. The role of C9orf72

10.8.1. Analysis of the role of C9orf72 on these endophenotypes was limited by the small number of participants who tested positive for the expansion. The characterization of cognition in asymptomatic C9orf72 gene carriers has often been limited by small sample sizes due to the rarity of this gene. This study found no differences between C9orf72 positive and negative ALS relatives on all cognitive tests except for semantic fluency, where contrary to previous studies, C9orf72 positive relatives performed better than C9orf72 negative relatives.

10.8.2. In a recent study of asymptomatic C9orf72 gene carriers (in a German cohort), C9orf72 positive carriers had significantly lower scores on phonemic fluency compared to non-family member controls (151). These deficits correlated reduced white matter integrity in inferior and orbitofrontal cortical areas. However, as was the case in this study, they found no significant difference between C9orf72 positive relatives and C9orf72 negative relatives on phonemic fluency.

10.8.3. Results suggested that C9orf72 is not the sole driver of the endophenotypes observed. This was also observed in aggregation studies, where higher rates of neuropsychiatric disease were seen in both C9orf72 positive and C9orf72 negative kindred (102,135).

10.9. Evaluation of endophenotype criteria

10.9.1. The extent to which candidate endophenotypes meet the Gottesman and Gould criteria are summarized in table 10.1. below. All outcomes are shown to be highly heritable according to twin studies, with heritability estimates ranging from 0.35 to 0.62. Verbal fluency and naming deficits are well known cognitive deficits associated with ALS. There is also converging evidence that ALS is associated with low openness to experience personality traits. The only evidence of an association between attention to detail and ALS comes from one aggregation study showing a higher incidence of autism in ALS kindred. There are currently no reported studies that indicate ALS is associated low conscientiousness traits. The results of thesis provide evidence that these putative endophenotypes are independent of clinical

state, co-segregate within families and are found at a higher rate in unaffected relatives.

Table 10.1. Endophenotype checklist according to Gottesman and Gould's criteria.

Endophenotype criteria	Verbal fluency	Confrontati onal naming	Attention to detail	Openness to experience	Conscientio usness
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

10.9.2. Another useful rubric for evaluating these endophenotypes is provided by Iacono et al. (412) best practice guidelines (see table 10.2.). This framework proposes three steps to validate an endophenotype and determine its usefulness. According to step 1, if a trait is associated with

associated with the illness, is highly heritable, is present in unaffected relatives and shares genetic variation with the clinical phenotype it can be considered a ‘candidate’ or ‘putative’ endophenotype. If the putative endophenotype is associated with specific gene variants and these variants are associated with the clinical phenotype the endophenotype loses the qualifier ‘candidate’ or ‘putative’ from the endophenotype status. The final step deals with the usefulness of an endophenotype, and how it can advance knowledge of the etiology of a disorder.

Table 10.2. Iacano's best practice guidelines for endophenotypes.

1. Putative endophenotype threshold criteria	1. Associated with one or more relevant clinical phenotype and
	2. Is heritable and/or
	3. Is present in first degree relatives of those with the clinical phenotype and/or
	4. Shares genetic variance with the clinical phenotype
2. Molecular genetic endophenotype verification	5. Shows verified association with specific gene variants
	6. These verified variants show robust association with the clinical phenotype
3. Utility	7. Predicts the development of the clinical phenotype
	8. Enhances theoretical understanding of the brain mechanism accounting for endophenotype individual differences
	9. Informs an animal model
	10. Identifies genetic variants that have relatively large effect

10.9.3. Based on this rubric, verbal fluency, confrontational naming, and low openness to experience can be considered ‘putative’ endophenotypes of

ALS. Evidence from this thesis and previous literature shows that they are associated with the illness, are highly heritable, and are present in unaffected relatives, however further research is required to determine if they share genetic variation with the clinical phenotype. Future research is also needed to evaluate if verbal fluency, confrontational naming, and low openness to experience can be molecularly and genetically verified, and if they can have utility in advancing our knowledge of the etiology of ALS.

10.10. Limitations

10.10.1. Due to the Covid-19 pandemic, recruitment for this project was cut short earlier than planned. While the study was able to achieve a sufficiently powered sample size for ALS relatives, this curtailment of recruitment did negatively affect the sample size of controls. Mid-way through the study it became clear that ALS relatives had a binomial age distribution, with a large portion of relatives being the siblings (usually in their 60's or 70's) or the offspring (usually in their 30's or 40's) of patients. In contrast, the controls who were recruited were often older, with a large proportion retirement aged. As a result, the controls were generally older than the ALS relative group. The final phase of control recruitment aimed to counteract this imbalance by targeting younger controls, providing a better age match. Unfortunately, as controls could not be recruited due to Covid-19 related restriction, this could not be accomplished.

10.10.2. This limitation was addressed in two ways. Neuropsychological performance was converted to z-scores using age-matched normative data provided in test manuals and published literature. As a result, each

participant's individual cognitive performance was scored relative to their age group. As normative data was not available for neuropsychiatric scores, an alternative method was applied. Multiple linear regressions were carried out with both age and group status (i.e., ALS relative or control) included in the model. Using this method, the effect of group status on neuropsychiatric traits scores could be examined, holding age constant.

10.10.3. In analyzing the effect of cognitive reserve on ALS, one of the key limitations was the lack of a control group. This would have allowed for an estimation of what change in cognition was due to practice effects and/or regression towards the mean. This study was also limited by the lack of neuroimaging or electrophysiological data to quantify pathological change. Ideally, future studies should implement longitudinal neuropsychological and neuroimaging/electrophysiological measurement. They should use control groups to estimate practice effects and regression to the mean and if significant attrition occurs, implement joint longitudinal modelling to maximize power.

10.10.4. Similarly, interpretation of the cluster analysis is limited by the lack of a control group and the high degree of missing data, resulting in listwise deletion of participants. Future studies are required to replicate and validate the existence of these sub-groups and determine their relation to phenotypes in patients.

10.11. Conclusions

10.11.1. Relatives of ALS patients display several neuropsychological and neuropsychiatric endophenotypes. The most robust cognitive

endophenotypes were deficits in verbal fluency and confrontational naming, which clustered in familial ALS relatives and were only partially attributable to IQ. The most robust neuropsychiatric endophenotypes were attention to detail autism traits, and low openness to experience and conscientiousness traits. These endophenotypes were present in both C9orf72 positive and C9orf72 negative participants, indicating that alternative, currently unknown risk genes are also driving this effect.

10.11.2. Cluster analysis of the neuropsychiatric and neuropsychological data identified two potential sub-groups of ALS relatives. Cluster 1 had impaired cognitive functioning and high levels of neuropsychiatric traits while cluster 2 had normal cognition, higher apathy and greater autism attention to detail traits. These clusters may represent diverging risk for ALS and related neuropsychiatric disease.

10.12. Implications for future research

10.12.1. The evaluation of alternative versions of the ECAS will facilitate longitudinal studies of cognition in ALS. This thesis established that the three versions of the ECAS are highly similar, though not strictly equivalent. Furthermore, and contrary to a previous Scottish study (35), there was evidence of subtle practice effects even when using alternative forms. These practice effects should be considered when measuring cognitive change over time and highlight the importance of a control group when carrying out longitudinal studies.

10.12.2. A two one sided t-tests (TOST) method was implemented to test the equivalency of the different ECAS versions. Previous methods would often

use standard t-tests and conclude equivalency if they failed to reject the null hypothesis. However, this does not directly assess equivalency. The TOST approach is advantageous in determining equivalency as it assumes that there is some degree of difference between tests and then tests this null hypothesis. Future studies of cognitive tests in ALS should similarly adopt the TOST approach to directly evaluate equivalency.

10.12.3. The ECAS equivalency study also provides two useful resources for clinical practice in Ireland. Firstly, Irish age and education derived abnormality clinical cut-offs for ECAS versions B and C (version A cut-offs are already published (248)). These can help clinicians to quickly and easily identify ALS patients with probable cognitive impairment and refer them for full neuropsychological assessment. Secondly, Reliable Change Indices (RCI) to interpret cognitive decline. The RCI cut-offs take into consideration the practice effects observed and provide cut-offs for clinically meaningful cognitive decline. These resources are now utilized in the Irish MND multidisciplinary clinic, helping in the reliable and valid screening of cognition in ALS.

10.12.4. The finding that cognitive reserve may offset cognitive impairment in ALS has a number of implications for future research. ALS is well known for being a heterogeneous disease, with a wide range of presentations. Cognitive reserve may explain why some ALS patients experience cognitive decline while others do not, as well as why some individuals have a more rapid cognitive decline. Cognitive reserve also offers a potential avenue for intervention. By promoting cognitively engaging activities, clinicians may

be able to delay the onset of cognitive symptoms in ALS patients. Future studies should also investigate the extent to which cognitive reserve impacts the neuropsychological and neuropsychiatric profile of unaffected relatives.

10.12.5. When carrying out the analysis of cognitive reserve in ALS, a relatively underutilized method of analysis, called joint models, was used. Joint models combine Cox survival models with linear mixed effects models, allowing us to account for data missing-at-random and data missing-not-at-random. This approach reduces the impact of attrition, which is particularly problematic in aggressive neurodegenerative diseases such as ALS. Future studies should incorporate joint modelling into their longitudinal analysis to enable more accurate estimates of cognitive decline over time.

10.12.6. The purpose of discovering endophenotypes of ALS is to facilitate the discovery of new ALS risk genes and elucidating the etiology of ALS. The endophenotype approach has proven successful in identifying risk genes in other conditions. For example, EEG endophenotypes and clinical diagnosis were used to identify GABRA2 and CHRM2 as novel risk genes for alcohol use disorder (413,414). Cognitive endophenotypes have successfully been applied in discovering ATXN7, CSMD1, DRD2, GRIN2A, GRIN3A, and GRM3 as risk genes for schizophrenia (164,415,416).

10.12.7. An endophenotype approach to gene discovery in ALS has recently begun. A recent GWAS (417) used both ALS diagnosis and psychiatric disease as outcomes, identifying several potentially pleiotropic risk loci, including CNNM2, SRGAP1, KRT18P55, SRGAP1, NCKAP5L, SPIRE1,

AS3MT and C9orf72. The future use of the putative endophenotypes identified in this thesis may help in the discovery of other risk genes for ALS.

10.12.8. If replicated and validated, these putative endophenotypes may help to improve diagnostic accuracy in ALS and help develop targeted interventions for at-risk individuals. Novel analytical techniques such as machine learning could build on the cluster analysis carried out in this thesis, to help identify the multi-modal nature of endophenotypes of ALS (418). These techniques would ideally incorporate neuropsychological, neuropsychiatric, neuroimaging and transcript-based measures to identify patterns of ALS risk.

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12. Appendix

12.1. Appendix 1: Family member information leaflet

Website: www.beaumont.ie

Ospidéal Beaumont



BEAUMONT HOSPITAL

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Family Member Information Leaflet

**Study title: A study of Multiple Effects of Genes Associated with MND
(Targeting Gene Pleiotropy: ALS Genes and Neuropsychiatric Endophenotypes)**

Principal investigator's name: Professor Orla Hardiman

Principal investigator's title: Consultant Neurologist

Telephone number of principal investigator: 01 896 4497

Data Controller's/joint Controller's Identity: Beaumont Hospital (Prof. Orla Hardiman)

Data Controller's/joint Controller's Contact Details: 01 896-4497

Data Protection Officer's Identity: Mr. Mark Graham

Data Protection Officer's Contact Details: dpo@beaumont.ie

You are being invited to take part in a clinical research study to be carried out at Beaumont Hospital.

Before you decide whether or not you wish to take part, you should read the information provided below carefully and, if you wish, discuss it with your family, friends or GP (doctor). Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you.

You don't have to take part in this study. If you decide not to take part it won't affect the future medical care of you or your family member.

You don't have to take part in this study. If you decide not to take part it won't affect your future medical care.

Why is this study being done?

Some people who have Motor Neurone Disease (MND) have trouble with thinking and memory, though not all. Our recent research has suggested that there can be more neurological, psychological and psychiatric conditions in family members of those with MND compared to the general population. This research study is taking place to find out if there are genetic factors which might explain this. We would also like to study the clinical similarities and differences between patients, controls (healthy individuals without MND) and their family members using MRI brain scanning.

Who is organising and funding this study?

The study is being done by Prof. Hardiman, consultant neurologist and Dr. Niall Pender, Principal Clinical Neuropsychologist and their teams, in association with collaborators from Trinity College Dublin. The project is funded through the Irish Motor Neurone Disease Association "Ice Bucket Challenge", Science Foundation Ireland and Research Motor Neurone.

Why am I being asked to take part?

You have been asked to participate in this study because your family member has MND, and we are comparing family members of people with MND with family

members of people who don't have MND. This is to find out whether we can identify differences across families that might provide clues as to why MND affects some families and not others.

How will the study be carried out?

The study began in June 2015 and will continue until sufficient information has been collected. Information will be collected on approximately 100 patients and 100 controls (individuals not diagnosed with MND) and their family members. The individuals taking part will fill out a questionnaire on their family history, give a blood sample, complete an online questionnaire and do some tests of thinking, memory and reasoning skills (called neuropsychological tests) at the participant's home and may also undergo an MRI scan and EEG in St James's Hospital.

What will happen to me if I agree to take part?

There are a series of different parts to the study which will take place over a number of days, at times and dates that are convenient to you. If you agree to take part in the study, you will be asked to do the following:

Complete a questionnaire on your family history. This will take place in your home and will take one hour to complete.

You will also be asked to donate a blood sample (approximately 10 mls/ 3 teaspoons).

You will be asked to complete neuropsychological tests on your memory, language and thinking (lasting less than 1-2 hours)

You will be asked to complete an online questionnaire on your thoughts and feelings. This will take approximately 30 minutes to complete.

At a later stage in our study, you may be asked to do the following:

An MRI will also take place in St. James's Hospital, which will require you to lie still for 45 minutes.

An EEG will take place in St. James's Hospital, which will involve you wearing a cap fitted with electrodes and take approximately 2 hours.

The study also requires a review of your medical records by a member of the clinical team.

To allow tracking of the progression of MND we hope to perform three separate evaluations separated by 4-month intervals.

What are the benefits?

There is no immediate benefit to you or your family in participating in this study. However, the information that will be obtained from this study will increase our knowledge of MND and may be of benefit to future patients.

What are the risks?

The blood sample is usually taken from a vein in the arm. The risks are minor; occasionally there can be minimal blood loss, swelling or tenderness at the insertion site.

The MRI scanner is a rather small enclosed space and it might cause some discomfort to lie still for 45 minutes. Ear plugs will be provided because the scanner produces a loud thumping noise.

Many of the participants have already had an MRI, but for those who have not, it is possible that the brain scan might identify an unexpected or previously unknown disease. It is also possible that some of the neuropsychological tests will reveal the presence of an unexpected finding. If this occurs, the new findings will be followed up by the clinical team and their GP will also be notified.

What if something goes wrong when I'm taking part in this study?

We do not anticipate that anything will go wrong. However, if at any time you feel that your participation in the study is unduly stressful, you are free to discontinue. This will not affect in any way the quality of care you or your family members receive at Beaumont Hospital.

Will it cost me anything to take part?

The study will not cost you anything. If you wish, we can reimburse your reasonable travel costs (up to €40) if you provide us with receipts.

What will happen to my blood sample?

DNA will be extracted from your blood sample and the samples will be coded. This codified information is stored in Trinity College. The code file is stored separately to the DNA. Access to your DNA is restricted to Prof. Hardiman and approved members of her team. Those analysing your DNA will not be able to identify you. The DNA will be used to try to find genes which increase and decrease the risk of developing MND.

The results of any genetic tests will **not** be made available to any party involved in your medical care, other than the research team. As this work is research based, we cannot provide you with the individual results of genetic studies performed on your DNA.

The samples will be stored indefinitely but future tests on your DNA, other than those specified, will require separate approval by the Beaumont Hospital Ethics (Medical Research) Committee.

All samples will be destroyed if you decide to withdraw from the study.

Is the study confidential?

All of the information collected from participating individuals will be kept strictly confidential. The investigators may request information on your condition from your GP and your medical records will be reviewed.

Data will be stored in password-protected encrypted files in Trinity College and the neuropsychological test information will be stored securely in the Academic Unit of Neurology, Trinity College Dublin. The data can only be accessed by Prof. Hardiman and approved members of her team. The data will be codified (each individual will be assigned a unique code) so that those analysing the data will not

be able to identify you and no personal information will be included in publications in the scientific literature or at scientific conferences.

Data generated during the study will be retained indefinitely to allow further analysis. It may be used in related studies in the future, subject to approval by the Beaumont Hospital Ethics (Medical Research) Committee.

Data Protection

We will be using your personal information in our research to help us to understand the cause of ALS, and to develop new and more effective treatments. The essential data being collected include demographics, neuropsychological, MRI and EEG data, and blood samples.

Your data is being processed in accordance with Article 6(1)(e) Public Interest; and Article (9)(2)(j) Scientific Research Purposes of the General Data Protection Regulation 2016.

Members of the project research team, under the approval and supervision of Prof. Orla Hardiman, Principal Investigator, will have access to your information. Your data in coded form will be shared with other European Motor Neurone Disease research groups who are carrying out similar projects and collecting similar information.

Your personal data will be coded using your unique identification number. Paper data will be retained for 5 years, until the study has concluded and then destroyed. Electronic data will be transferred to the Academic Unit of Neurology for analysis. Following completion of the study anonymised electronic data will be stored on a secure server in the Academic Unit of Neurology in Trinity College Dublin.

As your data will be coded at the time of testing with a unique identification number we do not foresee any situation where a data breach could cause harm. The electronic data on computers will also be coded and the key to link your name with the number will be kept separately. After completion of the study this key will be destroyed. In the unlikely event of a data breach, it will only be possible for someone to see your raw test scores which will not be meaningful to an untrained third party.

You have a right to withdraw your consent at any stage during the research study without giving a reason and without penalty. You can inform any member of the research team of your decision to withdraw at any time and they will accommodate your request.

You have the right to lodge a complaint with the Data Protection Commissioner about this research study if you feel your personal data is being mishandled.

You have a right to request access to your data and a copy of it, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to restrict or object to processing, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to have any inaccurate information about you corrected or deleted, unless their request would make it impossible or make it very difficult to conduct the research.

You have a right to have your personal data deleted, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to data portability, meaning you have a right to move your data from one controller to another in a readable format.

There will be no automated decision making or profiling using your data.

If automated decision making or profiling were to occur, you have the right to object to this if you wish.

You will be informed if we intend to further process your personal data and you will be provided with information on that other purpose.

You will be informed if we wish to transfer your data to a country outside of the EU or an international organisation and will be advised on the safeguards you have in place to protect your data. We do not foresee any situation where we will be transferring data outside the EU.

Consent to Future Uses

If you decide to take part in this study you will give your permission for your data to be used for this research study. We would like to ask your permission to store your data for possible future use in research. Deciding to consent to future use of your data is completely voluntary. If you decide to consent and change your mind, you can opt out at any time. You don't have to give us a reason. If you choose not to consent to future research, your regular care will not be affected.

MND is a rare disease and the data you provide in this study is valuable. Your stored data could be included in future research studies to make them bigger and more powerful. Your data may be used for future research projects carried out by a member of our research team or we may share your data with other organisations and researchers. Future research projects may be related to the present study (e.g. other studies that look at thinking and behaviour changes over

time in family members of people with MND) or unrelated (e.g. investigating thinking and behavioural changes in people with different conditions).

This project has a data controller (Professor Hardiman) to ensure we are looking after your data responsibly and using it properly. If you consent to the use of your data in future research studies your data will be assigned a number and will be identified only by this number. Data will be stored on a secure server in the Academic Unit of Neurology in Trinity College Dublin. Access to your data will be restricted to those approved by Professor Hardiman and would only be shared with research partners who act in accordance with recognised ethical standards for scientific research and who have obtained ethical approval to conduct research. The data controller will ensure that your data is completely and adequately protected in accordance with the requirements of the data protection laws applicable in the EU.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.

If you need any further information now or at any time in the future, please contact:

Name: Mr. Mark Heverin

Research Manager to Professor Hardiman

Address: Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin

152-160 Pearse Street, Dublin 2

Phone No (office hours only): 01 896 4376

Email: mark.heverin@tcd.ie

Name: Dr. Niall Pender

Principal Clinical Neuropsychologist/Head of Department

Address: Dept. of Psychology, Beaumont Hospital, Beaumont Rd, Dublin 9

Phone No (office hours only): 01 809 2414 / 01 809 2223

Name: Dr Marie Ryan Research Registrar Address: Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin 152-160 Pearse Street, Dublin 2 Phone No: 089 4515488 Email: ryanm65@tcd.ie	Name: Emmet Costello Research Assistant Address: Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin 152-160 Pearse Street, Dublin 2 Phone No: 089 2018386 Email: costele2@tcd.ie

12.2. Appendix 2: Family member consent form

Website: www.beaumont.ie

Ospidéal Beaumont



BEAUMONT HOSPITAL

P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Family Member Consent Form

**Study title: A study of Multiple Effects of Genes Associated with MND
(Targeting Gene Pleiotropy: ALS Genes and Neuropsychiatric Endophenotypes)**

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes ☐	No ☐
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes ☐	No ☐
I understand that I can withdraw my biological material at any time without any negative repercussions.	Yes ☐	No ☐
I understand that my biological material will be disposed of in a lawful and respectful way.	Yes ☐	No ☐
I am aware of the potential risks of this research study.	Yes ☐	No ☐
I give permission for researchers to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	Yes ☐	No ☐
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes ☐	No ☐
I consent to take part in this research study involving genetic testing having been fully informed of the risks, benefits and alternatives.	Yes ☐	No ☐

I give informed explicit consent to have my data processed as part of this research study.	Yes ☐	No ☐
I consent to give a DNA sample or samples for this research project. I understand that giving a DNA sample or samples for this research is my own decision.	Yes ☐	No ☐
I consent to be contacted by researchers as part of this research study.	Yes ☐	No ☐

FUTURE CONTACT		
I consent to be re-contacted by researchers about possible future research unrelated to the current study for which I may be eligible.	Yes ☐	No ☐

STORAGE AND FUTURE USE OF INFORMATION		
RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]		
OPTION 1: I give permission for my biological material/data to be stored for <u>possible future research related</u> to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 2: I give permission for my biological material/data to be stored for <u>possible future research related</u> to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3: I give permission for my biological material/data to be stored for <u>possible future research unrelated</u> to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 4: I give permission for my biological material/data to be stored for <u>possible future research unrelated</u> to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐

OPTION 5: I agree that some future research projects may be carried out by researchers working for <u>commercial/pharmaceutical companies</u> .	Yes ☐	No ☐
OPTION 6: I understand <u>I will not be entitled to a share of any profits</u> that may arise from the future use of my material/data or products derived from it.	Yes ☐	No ☐
DESTRUCTION OF RESEARCH MATERIAL [please choose one or more as you see fit]		
OPTION 1: I request that my <u>biological material be destroyed but</u> give permission for <u>data</u> derived from my biological material to be <u>stored</u> for <u>future research related</u> to the current study <u>only if consent is obtained</u> at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 2: I request that my <u>biological material be destroyed but</u> I give permission for my <u>data</u> derived from my biological material to be <u>stored</u> for <u>possible future research related</u> to the current study <u>without further consent</u> being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3: I request that my <u>biological material be destroyed but</u> give permission for <u>data</u> derived from my biological material to be <u>stored</u> for <u>future research unrelated</u> to the current study <u>only if consent is obtained</u> at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 4: I request that my <u>biological material be destroyed but</u> give permission for <u>data</u> derived from my biological material to be <u>stored</u> for <u>future research unrelated</u> to the current study <u>without further consent</u> being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 5: I request that all biological material/data previously collected can no longer be used by researchers and is destroyed.	Yes ☐	No ☐

| |

Patient Name (Block Capitals) | Patient Signature | Date

Translator Name (Block Capitals) Translator Signature
Date

Legal Representative/Guardian Name Legal Representative/Guardian Signature
Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

| | |

-----Name (Block Capitals) | Qualifications | Signature | Date

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

12.3. Appendix 3: Control information leaflet

Website: www.beaumont.ie

Ospidéal Beaumont



BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Control Information Leaflet

Study title: A study of Multiple Effects of Genes Associated with MND

Principal investigator's name: Professor Orla Hardiman

Principal investigator's title: Consultant Neurologist

Telephone number of principal investigator: 01 896 4497

Data Controller's/joint Controller's Identity: Beaumont Hospital (Prof. Orla Hardiman)

Data Controller's/joint Controller's Contact Details: 01 896-4497

Data Protection Officer's Identity: Mr. Mark Graham

Data Protection Officer's Contact Details: dpo@beaumont.ie

You are being invited to take part in a clinical research study to be carried out at Beaumont Hospital.

Before you decide whether or not you wish to take part, you should read the information provided below carefully and, if you wish, discuss it with your family, friends or GP (doctor). Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you.

You don't have to take part in this study. If you decide not to take part it won't affect your future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured it won't affect the quality of treatment you get in the future.

Why is this study being done?

Some people who have Motor Neurone Disease (MND) have trouble with thinking and memory, though not all. Our recent research has suggested that there can be more neurological, psychological and psychiatric conditions in family members of those with MND compared to the general population. This research study is taking place to find out if there are genetic factors which might explain this. We would also like to study the clinical similarities and differences between patients, controls (healthy individuals without MND) and their family members using MRI brain scanning.

Who is organising and funding this study?

The study is being done by Prof. Hardiman, consultant neurologist and Dr. Niall Pender, Principal Clinical Neuropsychologist and their teams, in association with collaborators from Trinity College Dublin. The project is funded through the Irish Motor Neurone Disease Association "Ice Bucket Challenge" , Science Foundation Ireland and Research Motor Neurone.

Why am I being asked to take part?

You have been asked to participate in this study because you do not have MND. Healthy individuals are needed for comparative purposes.

How will the study be carried out?

The study will begin in June 2015 and will continue until sufficient information has been collected. Information will be collected on approximately 100 patients and 100 controls (individuals not diagnosed with MND) and their family members. The individuals taking part will fill out a questionnaire on their family history, give a blood sample, complete an online questionnaire and do some tests of thinking, memory and reasoning skills (called neuropsychological tests) at the participant's home and also undergo an MRI scan and EEG in St James's Hospital.

What will happen to me if I agree to take part?

There are a series of different parts to the study which will take place over a number of days, at times and dates that are convenient to you. If you agree to take part in the study, you will be asked to do the following:

Complete a questionnaire on your family history. This will take place in your home and will take one hour to complete.

You will also be asked to donate a blood sample (approximately 10 mls/ 3 teaspoons).

You will be asked to complete neuropsychological tests on your memory, language and thinking (which take about 2 hours).

You will be asked to complete an online questionnaire on your thoughts and feelings. This will take approximately 30 minutes to complete.

At a later stage in our study, you may be asked to do the following:

An MRI will also take place in St. James's Hospital, which will require you to lie still for 45 minutes.

An EEG will take place in St. James's Hospital, which will involve you wearing a cap fitted with electrodes and take approximately 2 hours.

The study also requires a review of your medical records by a member of the clinical team.

To allow tracking of the progression of MND we hope to perform three separate evaluations separated by 4-month intervals.

What are the benefits?

There is no immediate benefit to you or your family in participating in this study. However, the information that will be obtained from this study will increase our knowledge of MND and may be of benefit to future patients.

What are the risks?

The blood sample is usually taken from a vein in the arm. The risks are minor; occasionally there can be minimal blood loss, swelling or tenderness at the insertion site.

The MRI scanner is a rather small enclosed space and it might cause some discomfort to lie still for 45 minutes. Ear plugs will be provided because the scanner produces a loud thumping noise.

Many of the participants have already had an MRI, but for those who have not, it is possible that the brain scan might identify an unexpected or previously unknown disease. It is also possible that some of the neuropsychological tests will reveal the presence of an unexpected finding. If this occurs, the new findings will be followed up by the clinical team and your GP will also be notified.

What if something goes wrong when I'm taking part in this study?

We do not anticipate that anything will go wrong. However, if at any time you feel that your participation in the study is unduly stressful, you are free to discontinue. This will not affect in any way the quality of care you receive at Beaumont Hospital.

Will it cost me anything to take part?

The study will not cost you anything. If you wish, we can reimburse your reasonable travel costs (up to €40) if you provide us with receipts.

What will happen to my blood sample?

DNA will be extracted from your blood sample and the samples will be coded. This codified information is stored in Trinity College. The code file is stored separately to the DNA. Access to your DNA is restricted to Prof. Hardiman and approved members of her team. Those analysing your DNA will not be able to identify you. The DNA will be used to try to find genes which increase and decrease the risk of developing MND. The results of any genetic tests will **not** be made available to any party involved in your medical care, other than the research team. As this work is research based, we cannot provide you with the individual results of genetic studies performed on your DNA.

The samples will be stored indefinitely but future tests on your DNA, other than those specified, will require separate approval by the Beaumont Hospital Ethics (Medical Research) Committee.

All samples will be destroyed if you decide to withdraw from the study.

Is the study confidential?

All of the information collected from participating individuals will be kept strictly confidential. The investigators may request information on your condition from your GP and your medical records will be reviewed.

Data will be stored in password-protected encrypted files in Trinity College and the neuropsychological test information will be stored securely in the Department of Psychology, Beaumont Hospital. The data can only be accessed by Prof. Hardiman and approved members of her team. The data will be codified (each individual will be assigned a unique code) so that those analysing the data will not be able to identify you and no personal information will be included in publications in the scientific literature or at scientific conferences.

Data generated during the study will be retained indefinitely to allow further analysis. It may be used in related studies in the future, subject to approval by the Beaumont Hospital Ethics (Medical Research) Committee.

Data Protection

We will be using your personal information in our research to help us to understand the cause of ALS, and to develop new and more effective treatments. The essential data being collected include demographics, neuropsychological and MRI and EEG data, and blood samples.

Your data is being processed in accordance with Article 6(1)(e) Public Interest; and Article (9)(2)(j) Scientific Research Purposes of the General Data Protection Regulation 2016.

Members of the project research team, under the approval and supervision of Prof. Orla Hardiman, Principal Investigator, will have access to your information. Your data in coded form will be shared with other European Motor Neurone Disease research groups who are carrying out similar projects and collecting similar information.

Your personal data will be coded using your unique identification number. Paper data will be retained for 5 years, until the study has concluded and then destroyed. Electronic data will be transferred to the Academic Unit of Neurology for analysis. Following completion of the study anonymised electronic data will be stored on a secure server in the Academic Unit of Neurology in Trinity College Dublin.

As your data will be coded at the time of testing with a unique identification number we do not foresee any situation where a data breach could cause harm. The electronic data on computers will also be coded and the key to link your name with the number will be kept separately. After completion of the study this key will be destroyed. In the unlikely event of a data breach, it will only be possible for someone to see your raw test scores which will not be meaningful to an untrained third party.

You have a right to withdraw your consent at any stage during the research study without giving a reason and without penalty. You can inform any member of the research team of your decision to withdraw at any time and they will accommodate your request.

You have the right to lodge a complaint with the Data Protection Commissioner about this research study if you feel your personal data is being mishandled.

You have a right to request access to your data and a copy of it, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to restrict or object to processing, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to have any inaccurate information about you corrected or deleted, unless their request would make it impossible or make it very difficult to conduct the research.

You have a right to have your personal data deleted, unless your request would make it impossible or make it very difficult to conduct the research.

You have a right to data portability, meaning you have a right to move your data from one controller to another in a readable format.

There will be no automated decision making or profiling using your data.

If automated decision making or profiling were to occur, you have the right to object to this if you wish.

You will be informed if we intend to further process your personal data and you will be provided with information on that other purpose.

You will be informed if we wish to transfer your data to a country outside of the EU or an international organisation and will be advised on the safeguards you have in place to protect your data. We do not foresee any situation where we will be transferring data outside the EU.

Consent to Future Uses

If you decide to take part in this study you will give your permission for your data to be used for this research study. We would like to ask your permission to store your data for possible future use in research. Deciding to consent to future use of

your data is completely voluntary. If you decide to consent and change your mind, you can opt out at any time. You don't have to give us a reason. If you choose not to consent to future research, your regular care will not be affected.

MND is a rare disease and the data you provide in this study is valuable. Your stored data could be included in future research studies to make them bigger and more powerful. Your data may be used for future research projects carried out by a member of our research team or we may share your data with other organisations and researchers. Future research projects may be related to the present study (e.g. other studies that look at thinking and behaviour changes over time in family members of people with MND) or unrelated (e.g. investigating thinking and behavioural changes in people with different conditions).

This project has a data controller (Professor Hardiman) to ensure we are looking after your data responsibly and using it properly. If you consent to the use of your data in future research studies your data will be assigned a number and will be identified only by this number. Data will be stored on a secure server in the Academic Unit of Neurology in Trinity College Dublin. Access to your data will be restricted to those approved by Professor Hardiman and would only be shared with research partners who act in accordance with recognised ethical standards for scientific research and who have obtained ethical approval to conduct research. The data controller will ensure that your data is completely and adequately protected in accordance with the requirements of the data protection laws applicable in the EU.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.

If you need any further information now or at any time in the future, please contact:

Name: Mr. Mark Heverin

Research Manager to Professor Hardiman

Address: Academic Unit of Neurology, Biomedical Sciences Institute, Trinity College Dublin

152-160 Pearse Street, Dublin 2

Phone No (office hours only): 01 896 4376

Email: mark.heverin@tcd.ie

Name: Dr. Niall Pender

Principal Clinical Neuropsychologist/Head of Department

Address: Dept. of Psychology, Beaumont Hospital, Beaumont Rd, Dublin 9

Phone No (office hours only): 01 809 2414 / 01 809 2223

Name: Emmet Costello

Research Assistant

Address: Academic Unit of Neurology, Biomedical Sciences Institute, Trinity
College Dublin

152-160 Pearse Street, Dublin 2

Phone No: 089 2018386

Email: costele2@tcd.ie

12.4. Appendix 4: Control consent form

Website: www.beaumont.ie

Ospidéal Beaumont



BEAUMONT HOSPITAL

P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

CONTROL CONSENT FORM

Study title: A study of Multiple Effects of Genes Associated with MND

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes ☐	No ☐
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes ☐	No ☐
I understand that I can withdraw my biological material at any time without any negative repercussions.	Yes ☐	No ☐
I understand that my biological material will be disposed of in a lawful and respectful way.	Yes ☐	No ☐
I am aware of the potential risks of this research study.	Yes ☐	No ☐
I give permission for researchers to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	Yes ☐	No ☐
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes ☐	No ☐
I consent to take part in this research study involving genetic testing having been fully informed of the risks, benefits and alternatives.	Yes ☐	No ☐

I give informed explicit consent to have my data processed as part of this research study.	Yes ☐	No ☐
I consent to give a DNA sample or samples for this research project. I understand that giving a DNA sample or samples for this research is my own decision.	Yes ☐	No ☐
I consent to be contacted by researchers as part of this research study.	Yes ☐	No ☐

FUTURE CONTACT		
I consent to be re-contacted by researchers about possible future research unrelated to the current study for which I may be eligible.	Yes ☐	No ☐

STORAGE AND FUTURE USE OF INFORMATION		
RETENTION OF RESEARCH MATERIAL IN THE FUTURE [please choose one or more as you see fit]		
OPTION 1: I give permission for my biological material/data to be stored for possible future research related to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 2: I give permission for my biological material/data to be stored for possible future research related to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3: I give permission for my biological material/data to be stored for possible future research unrelated to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 4: I give permission for my biological material/data to be stored for possible future research unrelated to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐

OPTION 5: I agree that some future research projects may be carried out by researchers working for commercial/pharmaceutical companies.	Yes ☐	No ☐
OPTION 6: I understand I will not be entitled to a share of any profits that may arise from the future use of my material/data or products derived from it.	Yes ☐	No ☐
DESTRUCTION OF RESEARCH MATERIAL [please choose one or more as you see fit]		
OPTION 1: I request that my biological material be destroyed but give permission for data derived from my biological material to be stored for future research related to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 2: I request that my biological material be destroyed but I give permission for my data derived from my biological material to be stored for possible future research related to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 3: I request that my biological material be destroyed but give permission for data derived from my biological material to be stored for future research unrelated to the current study only if consent is obtained at the time of the future research and the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 4: I request that my biological material be destroyed but give permission for data derived from my biological material to be stored for future research unrelated to the current study without further consent being required but only if the research is approved by a Research Ethics Committee.	Yes ☐	No ☐
OPTION 5: I request that all biological material/data previously collected can no longer be used by researchers and is destroyed.	Yes ☐	No ☐

| |

Patient Name (Block Capitals) | Patient Signature | Date

Translator Name (Block Capitals)
Date

Translator Signature

Legal Representative/Guardian Name
Date

Legal Representative/Guardian Signature

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

| | |

-----Name (Block Capitals)

| Qualifications | Signature | Date

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

12.5. Appendix 5: R code used in analysis

R code provided for ECAS analysis. Other neuropsychological outcomes were analysed using a similar code, with the relevant outcome measure in place. R code also supplied for PHQ-9 analysis. Analysis of other neuropsychiatric traits used similar code, with the relevant outcome variable specified.

```
## Cleaning ecas dataset ##
ecas_df <- read.delim("ecas_data.txt")

## Get recruited numbers ##
table(ecas_df$group)

## remove patients from dataframe ##
ecas_df <- subset(ecas_df, group != "Patient")

## remove under 18's ##
ecas_df <- subset(ecas_df, age >= 17)

## remove participants with dyslexia ##
ecas_df <- subset(ecas_df, id != "FH-F034-003")
ecas_df <- subset(ecas_df, id != "FH-F032-008")
ecas_df <- subset(ecas_df, id != "FH-F029-001")

## save the cleaned dataset ##
write.table(ecas_df, "ecas_data_cleaned.txt", sep = "\t")

## load cleaned dataset ##
ecas_df <- read.delim("ecas_data_cleaned.txt", header = TRUE)
options(scipen = 999)

##t-tests comparing relatives and controls ##
```

```

test1 <- t.test(ecas_total ~ group, data = ecas_df)
test2 <- t.test(ecas_alssp ~ group, data = ecas_df)
test3 <- t.test(als_nonsp ~ group, data = ecas_df)
test4 <- t.test(language ~ group, data = ecas_df)
test5 <- t.test(fluency ~ group, data = ecas_df)
test6 <- t.test(executive ~ group, data = ecas_df)
test7 <- t.test(memory ~ group, data = ecas_df)
test8 <- t.test(visuospatial ~ group, data = ecas_df)

##calculate effect size##
library(effectsize)
library(tidyverse)
library(rstatix)
library(ggpubr)
effectsize(test1)
effectsize(test2)
effectsize(test3)
effectsize(test5)
effectsize(test6)
effectsize(test7)

ecas_df %>% wilcox_effsize(language ~ group, paired = FALSE, ci = TRUE, conf.level = 0.95,
ci.type = "perc")

ecas_df %>% wilcox_effsize(visuospatial ~ group, paired = FALSE, ci = TRUE, conf.level = 0.95,
ci.type = "perc")

##ECAS total and fluency are significant. Fluency is significant after controlling for multiple
comparisons##

## Will run a non-parametric wilcox test to compare results ##

wtest1 <- wilcox.test(ecas_total ~ group, data = ecas_df)
wtest2 <- wilcox.test(ecas_alssp ~ group, data = ecas_df)
wtest3 <- wilcox.test(als_nonsp ~ group, data = ecas_df)
wtest4 <- wilcox.test(language ~ group, data = ecas_df)
wtest5 <- wilcox.test(fluency ~ group, data = ecas_df)
wtest6 <- wilcox.test(executive ~ group, data = ecas_df)
wtest7 <- wilcox.test(memory ~ group, data = ecas_df)
wtest8 <- wilcox.test(visuospatial ~ group, data = ecas_df)

```

```

median(ecas_df$ecas_total, na.rm = T)

## Check assumptions to ensure t-test is valid , note welch's t-test does not assume equal variance##
##check normal distribution using histogram and normality tests#
library(ggplot2)
library(pastecs)

#Create subsets of the two groups to explore assumptions##
relative_ecas <- subset(ecas_df, ecas_df$group == "Relative")
control_ecas <- subset(ecas_df, ecas_df$group == "Control")

by(ecas_df$ecas_total, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$ecas_alssp, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$sals_nonsp, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$language, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$fluency, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$executive, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$visuospatial, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)

## normality tests indicate normality assumption is violated. however, these tests are often
oversensitive in large sample sizes like this ##

by(ecas_df$ecas_total, ecas_df$group, hist)
qplot(sample = relative_ecas$ecas_total, stat="qq")
qplot(sample = control_ecas$ecas_total, stat="qq")
## Histograms look ok, qqplot indicates some kurtosis##

by(ecas_df$ecas_alssp, ecas_df$group, hist)
qplot(sample = relative_ecas$ecas_alssp, stat="qq")
qplot(sample = control_ecas$ecas_alssp, stat="qq")
## Histograms look ok, qqplot indicates kurtosis##

by(ecas_df$sals_nonsp, ecas_df$group, hist)
qplot(sample = relative_ecas$sals_nonsp, stat="qq")
qplot(sample = control_ecas$sals_nonsp, stat="qq")
## Histograms look ok, qqplot indicates kurtosis##

```

```

by(ecas_df$language, ecas_df$group, hist)
qplot(sample = relative_ecas$language, stat="qq")
qplot(sample = control_ecas$language, stat="qq")
##Histograms indicate severe deviation from normality, will need to use a non-parametric test##

by(ecas_df$fluency, ecas_df$group, hist)
qplot(sample = relative_ecas$fluency, stat="qq")
qplot(sample = control_ecas$fluency, stat="qq")
##Histograms indicate deviation from normality, will consider non-parametric test##

by(ecas_df$executive, ecas_df$group, hist)
qplot(sample = relative_ecas$executive, stat="qq")
qplot(sample = control_ecas$executive, stat="qq")
#### Histograms look ok, qqplot indicates some kurtosis##

by(ecas_df$memory, ecas_df$group, hist)
qplot(sample = relative_ecas$memory, stat="qq")
qplot(sample = control_ecas$memory, stat="qq")
## Histograms look ok, qqplot indicates significant kurtosis, consider non-parametric##

by(ecas_df$visuospatial, ecas_df$group, hist)
qplot(sample = relative_ecas$visuospatial, stat="qq")
qplot(sample = control_ecas$visuospatial, stat="qq")
##Histograms and qqplots indicate severe deviation from normality, will need to use a non-parametric
test##

##Overall language and visuospatial definitely violate assumption, will use nonparametric alternatives
for these two.

##Check assumptions of verbal fluency components##
by(ecas_df$spoken_vfi_s, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$spoken_words_s, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$spoken_time_s, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$spoken_score_s, ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(as.numeric(ecas_df$spoken_vfi_t), ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)
by(as.numeric(ecas_df$spoken_words_t), ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)

```

```

by(as.numeric(ecas_df$spoken_time_t), ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)

by(as.numeric(ecas_df$spoken_score_t), ecas_df$group, stat.desc, basic = FALSE, norm = TRUE)

## normality tests indicate normality assumption is violated. however, these tests are often
## oversensitive in large sample sizes like this.##

#

by(ecas_df$spoken_vfi_s, ecas_df$group, hist)
qplot(sample = relative_ecas$spoken_vfi_s, stat="qq")
qplot(sample = control_ecas$spoken_vfi_s, stat="qq")
## Histograms look ok, qqplot indicates kurtosis, consider non-parametric test##

by(ecas_df$spoken_words_s, ecas_df$group, hist)
qplot(sample = relative_ecas$spoken_words_s, stat="qq")
qplot(sample = control_ecas$spoken_words_s, stat="qq")
## Histograms look ok, qqplot is linear##

by(ecas_df$spoken_time_s, ecas_df$group, hist)
qplot(sample = relative_ecas$spoken_time_s, stat="qq")
qplot(sample = control_ecas$spoken_time_s, stat="qq")
## Histograms non-normal, qqplot indicates kurtosis, use non-parametric test##

by(ecas_df$spoken_score_s, ecas_df$group, hist)
qplot(sample = relative_ecas$spoken_score_s, stat="qq")
qplot(sample = control_ecas$spoken_score_s, stat="qq")
## Histograms look ok, qqplot indicates kurtosis, consider non-parametric test##

by(as.numeric(ecas_df$spoken_vfi_t), ecas_df$group, hist)
qplot(sample = as.numeric(relative_ecas$spoken_vfi_t), stat="qq")
qplot(sample = as.numeric(control_ecas$spoken_vfi_t), stat="qq")
## Histogram and qqplot non-normal, use non-parametric test##

by(as.numeric(ecas_df$spoken_words_t), ecas_df$group, hist)
qplot(sample = as.numeric(relative_ecas$spoken_words_t), stat="qq")
qplot(sample = as.numeric(control_ecas$spoken_words_t), stat="qq")
## Histogram and qqplot non-normal, use non-parametric test##

by(as.numeric(ecas_df$spoken_time_t), ecas_df$group, hist)

```

```

qqplot(sample = as.numeric(relative_ecas$spoken_time_t), stat="qq")
qqplot(sample = as.numeric(control_ecas$spoken_time_t), stat="qq")
## Histograms and qq-plot not normal for controls, use non-parametric test##

by(as.numeric(ecas_df$spoken_score_t), ecas_df$group, hist)
qqplot(sample = as.numeric(relative_ecas$spoken_score_t), stat="qq")
qqplot(sample = as.numeric(control_ecas$spoken_score_t), stat="qq")
## Histograms look ok, qqplot indicates kurtosis, consider non-parametric test##

##Overall spoken_time_s, spoken_vfi_t, spoken_words_t and spoken_time_t definitely violate
assumption, will use nonparametric alternatives for these four##

##### Compare Familial and Sporadic ALS with controls #####
##Test assumptions using levenes test##
library(car)
leveneTest(ecas_df$ecas_total, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$ecas_alssp, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$sals_nonsp, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$language, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$fluency, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$executive, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$memory, ecas_df$fam_hx, center = median)
leveneTest(ecas_df$visuospatial, ecas_df$fam_hx, center = median)
##Levene's test suggest Fluency has unequal variance##

##Run anovas##
anova1 <- aov(ecas_total ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova2 <- aov(ecas_alssp ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova3 <- aov(als_nonsp ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova4 <- aov(language ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova5 <- aov(fluency ~ fam_hx2, data = ecas_df, na.action = na.exclude)
kw_test5 <- kruskal.test(fluency ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova6 <- aov(executive ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova7 <- aov(memory ~ fam_hx2, data = ecas_df, na.action = na.exclude)
anova8 <- aov(visuospatial ~ fam_hx2, data = ecas_df, na.action = na.exclude)

summary(anova1)

```

```

summary(anova2)
summary(anova3)
summary(anova4)
summary(anova5)
summary(kw_test5)
summary(anova6)
summary(anova7)
summary(anova8)

##Check assumptions by plotting
plot(anova1) # Some kurtosis, residuals normal #
plot(anova2) # Some kurtosis, residuals normal #
plot(anova3) # Some kurtosis, residuals normal #
plot(anova4) # Strong kurtosis, residuals normal #
plot(anova5) # Strong kurtosis, residuals normal #
plot(anova6) # Little kurtosis, residuals normal #
plot(anova7) # Some kurtosis, residuals normal #
plot(anova8) # Strong kurtosis, residuals normal #

##Overall, assumptions appear to be upheld, could consider K-W test for verbal fluency##

##Conduct post-hoc tests##
post_hoc1 <- pairwise.t.test(ecas_df$ecas_total, ecas_df$fam_hx2, p.adjust.method = "BH")
post_hoc2 <- pairwise.t.test(ecas_df$ecas_alssp, ecas_df$fam_hx2, p.adjust.method = "BH")
library(pgirmess)
post_hoc3 <- kruskalmc(flucency ~ fam_hx2, data = ecas_df)

flucency_plot_2 <- ggplot(ecas_df, aes(fam_hx2, flucency, fill = fam_hx2))
flucency_plot_2 + geom_boxplot(size = 1.2) + geom_point() + geom_jitter() +
  labs(x = "Group", y = "Verbal Fluency") + theme_classic()

## Compare C9orf72 positive and negative participants##
t.test(age ~ c9_status_3, data = ecas_df)
t.test(education ~ c9_status_3, data = ecas_df)

```



```

c9_test1 <- t.test(ecas_total ~ c9_status_3, data = ecas_df)
c9_test2 <- t.test(ecas_alssp ~ c9_status_3, data = ecas_df)
c9_test3 <- t.test(als_nonsp ~ c9_status_3, data = ecas_df)
c9_test4 <- t.test(language ~ c9_status_3, data = ecas_df)
wc9_test4 <- wilcox.test(language ~ c9_status_3, data = ecas_df)
c9_test5 <- t.test(fluency ~ c9_status_3, data = ecas_df)
wc9_test5 <- wilcox.test(fluency ~ c9_status_3, data = ecas_df)
c9_test6 <- t.test(executive ~ c9_status_3, data = ecas_df)
c9_test7 <- t.test(memory ~ c9_status_3, data = ecas_df)
c9_test8 <- t.test(visuospatial ~ c9_status_3, data = ecas_df)
wc9_test8 <- wilcox.test(visuospatial ~ c9_status_3, data = ecas_df)

by(ecas_df$ecas_total, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$ecas_alssp, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$als_nonsp, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$language, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$fluency, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$executive, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)
by(ecas_df$visuospatial, ecas_df$c9_status_3, stat.desc, basic = FALSE, norm = TRUE)

## normality tests indicate normality assumption is violated. however, these tests are often
oversensitive in large sample sizes like this.##

#

c9_pos <- subset(ecas_df, ecas_df$c9_status_3 == "Positive")
c9_neg <- subset(ecas_df, ecas_df$c9_status_3 == "Negative")

by(ecas_df$ecas_total, ecas_df$c9_status_3, hist)
qqplot(sample = c9_pos$ecas_total, stat="qq")
qqplot(sample = c9_neg$ecas_total, stat="qq")
## Histograms look ok, qqplot indicates some kurtosis##

by(ecas_df$ecas_alssp, ecas_df$c9_status_3, hist)
qqplot(sample = c9_pos$ecas_alssp, stat="qq")
qqplot(sample = c9_neg$ecas_alssp, stat="qq")
## Histograms look ok, qqplot indicates some kurtosis##

```

```

by(ecas_df$sals_nosp, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$sals_nosp, stat="qq")
qplot(sample = c9_neg$sals_nosp, stat="qq")
## Histograms look ok, qqplot indicates kurtosis##

by(ecas_df$language, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$language, stat="qq")
qplot(sample = c9_neg$language, stat="qq")
##Histograms indicate severe deviation from normality, will need to use a non-parametric test##

by(ecas_df$fluency, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$fluency, stat="qq")
qplot(sample = c9_neg$fluency, stat="qq")
##Histograms indicate deviation from normality, some kurtosis, will consider non-parametric test##

by(ecas_df$executive, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$executive, stat="qq")
qplot(sample = c9_neg$executive, stat="qq")
#### Histograms look ok, qqplot indicates some kurtosis##

by(ecas_df$memory, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$memory, stat="qq")
qplot(sample = c9_neg$memory, stat="qq")
## Histograms look ok, qqplot indicates significant kurtosis, consider non-parametric##

by(ecas_df$visuospatial, ecas_df$c9_status_3, hist)
qplot(sample = c9_pos$visuospatial, stat="qq")
qplot(sample = c9_neg$visuospatial, stat="qq")
##Histograms and qqplots indicate severe deviation from normality, will need to use a non-parametric
test##

##Overall, language and visuospatial definitely violate normality assumption, may also use non-
parametric test for verbal fluency ##

##plot
library(scales)
library(ggplot2)

```

```

library(ggforce)
library(ggpubr)

my_comparisons2 <- list( c("Control", "Relative"))

fluency_test <- compare_means(fluency ~ group, comparisons = my_comparisons2, method = "t.test",
data = ecas_df)

fluency_test <- fluency_test %>% mutate(y.position = c(28))

fluency_plot <- ggplot(ecas_df, aes(group, fluency, fill = group))
fluency_plot + geom_boxplot(width = .3) + geom_jitter(alpha = .25, width = .2) +
  labs(x = "Group", y = "ECAS Verbal Fluency") + theme_classic() +
  theme(legend.position = "none") +
  theme(axis.title.x = element_text(vjust = 0, size = 14),
        axis.title.y = element_text(vjust = 2, size = 14)) +
  theme(axis.text = element_text(size = 12),
        axis.text.x = element_text()) + stat_compare_means(method = "t.test", label.x = 1.25, label.y = 40)

fluency_plot <- ggboxplot(ecas_df, x = "group", y = "fluency", fill = "group", width = .3) +
stat_pvalue_manual(fluency_test, label = "p.adj", hide.ns = TRUE) +

  labs(x = "Group", y = "Verbal fluency") + theme_classic() + geom_jitter(alpha = .25, width = .2) +

  theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +

  theme(axis.text = element_text(size = 12),
        axis.text.x = element_text()) + theme(legend.position = "none")

##Compare unrestricted and restricted fluency scores##
vf_test1 <- t.test(spoken_score_s ~ group, data = ecas_df)
vf_test2 <- t.test(spoken_score_t ~ group, data = ecas_df)
vf_test3 <- t.test(spoken_vfi_s ~ group, data = ecas_df)
vf_test4 <- t.test(spoken_words_s ~ group, data = ecas_df)
vf_test5 <- t.test(spoken_time_s ~ group, data = ecas_df)
wvf_test5 <- wilcox.test(spoken_time_s ~ group, data = ecas_df)
vf_test6 <- t.test(as.numeric(spoken_vfi_t) ~ group, data = ecas_df)
wvf_test6 <- wilcox.test(as.numeric(spoken_vfi_t) ~ group, data = ecas_df)
vf_test7 <- t.test(as.numeric(spoken_words_t) ~ group, data = ecas_df)
wvf_test7 <- wilcox.test(as.numeric(spoken_words_t) ~ group, data = ecas_df)
vf_test8 <- t.test(as.numeric(spoken_time_t) ~ group, data = ecas_df)

```

```

wvf_test8 <- wilcox.test(as.numeric(spoken_time_t) ~ group, data = ecas_df)

effectsize(vf_test1)
effectsize(vf_test2)
effectsize(vf_test3)
effectsize(vf_test4)

ecas_df %>% wilcox_effsize(spoken_time_s ~ group, paired = FALSE, ci = TRUE, conf.level = 0.95,
ci.type = "perc")

ecas_df %>% wilcox_effsize(spoken_vfi_t ~ group, paired = FALSE, ci = TRUE, conf.level = 0.95,
ci.type = "perc")

ecas_df %>% wilcox_effsize(spoken_words_t ~ group, paired = FALSE, ci = TRUE, conf.level =
0.95, ci.type = "perc")

ecas_df %>% wilcox_effsize(spoken_time_t ~ group, paired = FALSE, ci = TRUE, conf.level = 0.95,
ci.type = "perc")

## effect size of anovas##
omega_squared(anova1)
omega_squared(anova2)
omega_squared(anova1)

#plot groups
my_comparisons <- list( c("Control", "FALS"), c("Control", "SALS"), c("FALS", "SALS"))

ecas_ggtest1 <- compare_means(ecas_total ~ fam_hx2, comparisons = my_comparisons, method =
"t.test", p.adjust.method = "BH", data = ecas_df)

ecas_ggtest1 <- alssp_ggtest2 %>% mutate(y.position = c(140, 150, 160))

alssp_ggtest2 <- compare_means(ecas_alssp ~ fam_hx2, comparisons = my_comparisons, method =
"t.test", p.adjust.method = "BH", data = ecas_df)

alssp_ggtest2 <- alssp_ggtest2 %>% mutate(y.position = c(105, 110, 115))

fluency_ggtest3 <- compare_means(ecas_alssp ~ fam_hx2, comparisons = my_comparisons,
p.adjust.method = "BH", data = ecas_df)

fluency_ggtest3 <- fluency_ggtest3 %>% mutate(y.position = c(25, 30, 35))

fluency_ggtest3 <- scientific(fluency_ggtest3)

ecas_total_plot <- ggboxplot(ecas_df, x = "fam_hx2", y = "ecas_total", fill = "fam_hx2",width = .3) +
stat_pvalue_manual(ecas_ggtest1, label = "p.adj", hide.ns = TRUE) +

labs(x = "Group", y = "ECAS total") + theme_classic() +

```

```

theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +

theme(axis.text = element_text(size = 12),

      axis.text.x = element_text()) + stat_compare_means(method = "anova", label.x = 2, label.y =
150) + theme(legend.position = "none")

ecas_alssp_plot2 <- ggboxplot(ecas_df, x = "fam_hx2", y = "ecas_alssp", fill = "fam_hx2", width = .3)
+ stat_pvalue_manual(alssp_ggtest2, label = "p.adj", hide.ns = TRUE) +

labs(x = "Group", y = "ECAS ALS specific") + theme_classic() +

theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +

theme(axis.text = element_text(size = 12),

      axis.text.x = element_text()) + stat_compare_means(method = "anova", label.x = 2, label.y =
110) + theme(legend.position = "none")

ecas_alssp_plot3 <- ggboxplot(ecas_df, x = "fam_hx2", y = "fluency", fill = "fam_hx2", width = .3) +
stat_pvalue_manual(fluency_ggtest3, label = "p.adj", hide.ns = TRUE) +

labs(x = "Group", y = "Verbal fluency") + theme_classic() +

theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +

theme(axis.text = element_text(size = 12),

      axis.text.x = element_text()) + stat_compare_means(method = "kruskal.test", label.x = 2, label.y
= 28) + theme(legend.position = "none")

```

R Markdown code

```

---

title: "ecas analysis"
author: "Emmet Costello"
date: "March 10, 2021"
output: html_document

---

```${r setup, include=FALSE}

knitr::opts_chunk$set(echo = TRUE)

library(tidyverse)

library(readxl)

```

```

library(here)
library(knitr)
library(kableExtra)
library(summarytools)
library(table1)
library(MatchIt)

ecas_df <- here::here("ecas_data_cleaned.csv") %>% readr::read_csv()
relative_ecas <- subset(ecas_df, ecas_df$group == "Relative")
control_ecas <- subset(ecas_df, ecas_df$group == "Control")

...

```{r, results='asis'}
table1(~ gender + age + handedness + education + education_level + c9_status_2 + fam_hx2| group,
data=ecas_df,
      droplevels=F, overall=T)
...

```{r, results='asis'}
##create t-tests##

test1 <- t.test(ecas_total ~ group, data = ecas_df)

test2 <- t.test(ecas_alssp ~ group, data = ecas_df)

test3 <- t.test(als_nosp ~ group, data = ecas_df)

test4 <- t.test(language ~ group, data = ecas_df)
wtest4 <- wilcox.test(language ~ group, data = ecas_df)

test5 <- t.test(fluency ~ group, data = ecas_df)

test6 <- t.test(executive ~ group, data = ecas_df)

test7 <- t.test(memory ~ group, data = ecas_df)

```

```

test8 <- t.test(visuospatial ~ group, data = ecas_df)
wtest8 <- wilcox.test(visuospatial ~ group, data = ecas_df)

##round digits to two decimal spaces##
test1 = format(test1, digits = 3)
test2 = format(test2, digits = 3)
test3 = format(test3, digits = 3)
test4 = format(test4, digits = 3)
wtest4 = format(wtest4, digits = 3)
test5 = format(test5, digits = 3)
test6 = format(test6, digits = 3)
test7 = format(test7, digits = 3)
test8 = format(test8, digits = 3)
wtest8 = format(wtest8, digits = 3)

##make blank table##
table = matrix(NA, nrow = 8, ncol = 6)

##make column names#
colnames(table) = c("ECAS score", "Control", "Relative", "t/W", "df", "p")

##add information to table##
table[1,] = c("ECAS total", mean(control_ecas$ecas_total, na.rm = T), mean(relative_ecas$ecas_total,
na.rm = T, digits = 2), test1["statistic"], test1["parameter"], test1["p.value"])

table[2,] = c("ALS specific", mean(control_ecas$ecas_alssp, na.rm = T),
mean(relative_ecas$ecas_alssp, na.rm = T, digits = 2), test2["statistic"], test2["parameter"],
test2["p.value"])

table[3,] = c("ALS non specific", mean(control_ecas$sals_nonsp, na.rm = T),
mean(relative_ecas$sals_nonsp, na.rm = T), test3["statistic"], test3["parameter"], test3["p.value"])

table[4,] = c("Language", median(control_ecas$language, na.rm = T),
median(relative_ecas$language, na.rm = T), wtest4["statistic"], NA, wtest4["p.value"])

table[5,] = c("Verbal fluency", mean(control_ecas$f fluency, na.rm = T), mean(relative_ecas$f fluency,
na.rm = T), test5["statistic"], test5["parameter"], test5["p.value"])

```

```

table[6,] = c("Executive", mean(control_ecas$executive, na.rm = T), mean(relative_ecas$executive,
na.rm = T), test6["statistic"], test6["parameter"], test6["p.value"])

table[7,] = c("Memory", mean(control_ecas$memory, na.rm = T), mean(relative_ecas$memory,
na.rm = T), test7["statistic"], test7["parameter"], test7["p.value"])

table[8,] = c("Visuospatial", median(control_ecas$visuospatial),
median(relative_ecas$visuospatial), wtest8["statistic"], NA, wtest8["p.value"])

...

```{r make a table, results='asis'}

kable(table, caption = "ECAS performance in Relatives vs Controls") %>%
  kableExtra::kable_styling(bootstrap_options = "striped", full_width = FALSE)

...

```{r, results='asis'}

##Plot a graph of the Verbal fluency scores ##

library(ggplot2)

fluency_plot <- ggplot(ecas_df, aes(group, fluency, fill = group))

fluency_plot + geom_boxplot(size = 1.2) + geom_point() + geom_jitter() + labs(x = "Group", y =
"Verbal Fluency") + theme_classic()

...

```{r}

##Compare unrestricted and restricted fluency scores##

vf_test1 <- t.test(spoken_score_s ~ group, data = ecas_df)
vf_test2 <- t.test(spoken_score_t ~ group, data = ecas_df)

vf_test3 <- t.test(spoken_vfi_s ~ group, data = ecas_df)
vf_test4 <- t.test(spoken_words_s ~ group, data = ecas_df)
vf_test5 <- t.test(spoken_time_s ~ group, data = ecas_df)
wvf_test5 <- wilcox.test(spoken_time_s ~ group, data = ecas_df)

```



```

vf_test6 <- t.test(as.numeric(spoken_vfi_t) ~ group, data = ecas_df)
wvf_test6 <- wilcox.test(as.numeric(spoken_vfi_t) ~ group, data = ecas_df)
vf_test7 <- t.test(as.numeric(spoken_words_t) ~ group, data = ecas_df)
wvf_test7 <- wilcox.test(as.numeric(spoken_words_t) ~ group, data = ecas_df)
vf_test8 <- t.test(as.numeric(spoken_time_t) ~ group, data = ecas_df)
wvf_test8 <- wilcox.test(as.numeric(spoken_time_t) ~ group, data = ecas_df)

##round digits to two decimal spaces##
vf_test1 = format(vf_test1, digits = 3)
vf_test2 = format(vf_test2, digits = 3)
vf_test3 = format(vf_test3, digits = 3)
vf_test4 = format(vf_test4, digits = 3)
vf_test5 = format(vf_test5, digits = 3)
wvf_test5 = format(wvf_test5, digits = 3)
vf_test6 = format(vf_test6, digits = 3)
wvf_test6 = format(wvf_test6, digits = 3)
vf_test7 = format(vf_test7, digits = 3)
wvf_test7 = format(wvf_test7, digits = 3)
vf_test8 = format(vf_test8, digits = 3)
wvf_test8 = format(wvf_test8, digits = 3)

##Create table comparing groups##
##make blank table##
table2 = matrix(NA, nrow = 8, ncol = 6)

##make column names#
colnames(table2) = c("Verbal fluency component", "Controls", "Relatives", "t/W", "df", "p")

##add information to table##

table2[1, ] = c("Verbal fluency score", mean(control_ecas$spoken_score_s, na.rm = T),
mean(relative_ecas$spoken_score_s, na.rm = T, digits = 2), vf_test1["statistic"],
vf_test1["parameter"], vf_test1["p.value"])

table2[2, ] = c("Verbal fluency index (vfi)", mean(control_ecas$spoken_vfi_s, na.rm = T),
mean(relative_ecas$spoken_vfi_s, na.rm = T, digits = 2), vf_test3["statistic"], vf_test3["parameter"],
vf_test3["p.value"])

table2[3, ] = c("Total words", mean(control_ecas$spoken_words_s, na.rm = T),
mean(relative_ecas$spoken_words_s, na.rm = T), vf_test4["statistic"], vf_test4["parameter"],
vf_test4["p.value"])

```

```
table2[4, ] = c("Time to read words", median(control_ecas$spoken_time_s, na.rm = T),
median(relative_ecas$spoken_time_s, na.rm = T), wvf_test5["statistic"], NA, vf_test5["p.value"])
```

```
table2[5, ] = c("Verbal fluency score", mean(as.numeric(control_ecas$spoken_score_t), na.rm = T),
mean(as.numeric(relative_ecas$spoken_score_t), na.rm = T), vf_test2["statistic"],
vf_test2["parameter"], vf_test2["p.value"])
```

```
table2[6, ] = c("Verbal fluency index (vfi)", median(as.numeric(control_ecas$spoken_vfi_t), na.rm =
T), median(as.numeric(relative_ecas$spoken_vfi_t), na.rm = T), wvf_test6["statistic"], NA,
wvf_test6["p.value"])
```

```
table2[7, ] = c("Total words", median(as.numeric(control_ecas$spoken_words_t), na.rm = T),
median(as.numeric(relative_ecas$spoken_words_t), na.rm = T), wvf_test7["statistic"], NA,
wvf_test7["p.value"])
```

```
table2[8, ] = c("Time to read words", median(as.numeric(control_ecas$spoken_time_t), na.rm = T),
median(as.numeric(relative_ecas$spoken_time_t), na.rm = T), wvf_test8["statistic"], NA,
wvf_test8["p.value"])
```

```
---
```

```
```{r table 2, results='asis'}
```

```
kable(table2, caption = "Components of verbal fluency performance: relatives vs controls") %>%
```

```
 kableExtra::kable_styling(bootstrap_options = "striped", full_width = FALSE)
```

```

```

```
```{r}
```

```
##Compare Familial and Sporadic Relatives to controls##
```

```
anova1 <- aov(ecas_total ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova2 <- aov(ecas_alssp ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova3 <- aov(als_nonsp ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova4 <- aov(language ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova5 <- aov(fluency ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
kw_test5 <- kruskal.test(fluency ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova6 <- aov(executive ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova7 <- aov(memory ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
anova8 <- aov(visuospatial ~ fam_hx2, data = ecas_df, na.action = na.exclude)
```

```
fluency_plot_2 <- ggplot(ecas_df, aes(fam_hx2, fluency, fill = fam_hx2))
```

```
fluency_plot_2 + geom_boxplot(size = 1.2) + geom_point() + geom_jitter() +  
  labs(x = "Group", y = "Verbal Fluency") + theme_classic()  
  
post_hoc1 <- pairwise.t.test(ecas_df$ecas_total, ecas_df$fam_hx2, p.adjust.method = "BH")  
post_hoc2 <- pairwise.t.test(ecas_df$ecas_alssp, ecas_df$fam_hx2, p.adjust.method = "BH")  
library(pgirmess)  
post_hoc3 <- kruskalmc(fluency ~ fam_hx2, data = ecas_df)  
...
```

R code for analysis of neuropsychiatric traits

```
## Load packages and dataset
library(tidyverse)
library(readxl)
library(here)
library(summarytools)
library(car)
library(effectsize)
library(rstatix)
library(ggpubr)
library(pgirmess)

traits_df <- read_excel("traits_data.xlsx", sheet = "summary")

## Remove patients
traits_df <- subset(traits_df, group_1 != "Patient")
traits_df <- subset(traits_df, group_2 != "NA")

## Change age and education to numeric
traits_df$age <- as.numeric(traits_df$age)
traits_df$education <- as.numeric(traits_df$education)
options(scipen = 999)

## Create groups to check assumptions

relative_df <- subset(traits_df, traits_df$group_1 == "Relative")
control_df <- subset(traits_df, traits_df$group_1 == "Control")
c9pos_df <- subset(traits_df, c9_status_2 == "Positive")
c9neg_df <- subset(traits_df, c9_status_2 == "Negative")

## Compare relatives and controls on PHQ-9 score
phq_test1 <- t.test(phq_total ~ group_1, data = traits_df)
phq_wtest <- wilcox.test(phq_total ~ group_1, data = traits_df)
effectsize(phq_test1)
wilcox_effsize(traits_df, phq_total ~ group_1, paired = FALSE, ci = TRUE)
```

```

##check assumptions

by(traits_df$phq_total, traits_df$group_1, hist) #Strong positive skew
qplot(sample = relative_df$phq_total, stat="qq") #Moderate kurtosis
qplot(sample = control_df$phq_total, stat="qq") #Moderate kurtosis

## Graph mean differences between relatives and controls
#Make tibble of adjusted p-values
stat.test <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "Control", "Relative", .009)

phq_plot_1 <- ggboxplot(traits_df, x = "group_1", y = "phq_total", fill = "group_1", width = .4) +
  stat_pvalue_manual(stat.test, y.position = 18, step.increase = 0.1, label = "p.adj", hide.ns = TRUE) +
  labs(x = "Group", y = "PHQ-9 total score") + theme_classic() +
  theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +
  theme(axis.text = element_text(size = 12),
        axis.text.x = element_text()) + theme(legend.position = "none")

phq_plot_1 + geom_jitter(alpha = .25, width = .2) + geom_violin(aes(fill = group_1), size = 1, alpha =
.2, adjust = 1.5)

## Run chi-square to compare the categorical PHQ variable
##create new phq variable for scores >= 10
traits_df$phq_cat3 <- ifelse(traits_df$phq_total >= 10, c("abnormal"), c("normal"))
chisq.test(traits_df$group_1, traits_df$phq_cat3)

## Compare FALS, SALS and Controls
##check homogeneity of variance
leveneTest(traits_df$phq_total, traits_df$group_2, center = median) #non sig

##Run anova
phq_anova1 <- aov(phq_total ~ group_2, data = traits_df)
summary(phq_anova1)

##check assumptions

```

```

plot(phq_anova1) ## Moderate skew on qq plot

##Run Kruskal Wallis test
phq_kw1 <- kruskal.test(phq_total ~ group_2, data = traits_df)
post_hoc1 <- kruskalmc(phq_total ~ group_2, data = traits_df)

##Plot differences

#Make tibble of adjusted p-values
stat.test <- tibble::tribble(
  ~group1, ~group2, ~p.adj,
  "Control", "FALS", .05,
  "Control", "SALS", .10,
  "FALS", "SALS", .10)

phq_plot_2 <- ggboxplot(traits_df, x = "group_2", y = "phq_total", fill = "group_2", width = .4) +
  stat_pvalue_manual(stat.test, y.position = 18, step.increase = 0.1, label = "p.adj", hide.ns = TRUE) +
  labs(x = "Group", y = "PHQ-9 total score") + theme_classic() +
  stat_compare_means(method = "kruskal.test", label.x = 2, label.y = 20) +
  theme(axis.title.x = element_text(vjust = 0, size = 14), axis.title.y = element_text(vjust = 2, size =
14)) +
  theme(axis.text = element_text(size = 12),
        axis.text.x = element_text()) + theme(legend.position = "none")

phq_plot_2 + geom_jitter(alpha = .25, width = .2) + geom_violin(aes(fill = group_2), size = 1, alpha =
.2, adjust = 1.5)

##Compare FALS, SALS and controls on PHQ categories
chisq.test(traits_df$group_2, traits_df$phq_cat3)

##Compare C9+ and C9- relatives on phq score
c9_phq_test1 <- t.test(phq_total ~ c9_status_2, data = traits_df)
c9_phq_wtest1 <- wilcox.test(phq_total ~ c9_status_2, data = traits_df)

##Check assumptions
by(traits_df$phq_total, traits_df$c9_status_2, hist) #Strong positive skew
qplot(sample = c9pos_df$phq_total, stat="qq") #Moderate kurtosis

```

```
qqplot(sample = c9neg_df$phq_total, stat="qq") #Moderate kurtosis
```

```
chisq.test(traits_df$c9_status_2, traits_df$phq_cat3)
```

```
table(traits_df$c9_status_2, traits_df$phq_cat3)
```

```
##Run a regression to control for age differences
```

```
model1 <- lm(phq_total ~ age + group_1, traits_df)
```

```
summary(model1)
```

```
plot(model1)
```

```
model2 <- lm(phq_total ~ age + group_2, traits_df)
```

```
summary(model2)
```

```
plot(model2)
```

```
model3 <- lm(phq_total ~ age + c9_status_2, traits_df)
```

```
summary(model3)
```

```
plot(model3)
```

R code for analysis of differential deficits, i.e., controlling for IQ.

```
library(tidyverse)
library(readxl)
library(here)
library(QuantPsyc)

## load cleaned dataset ##
neuropsych_df <- here::here("neuropsych_cleaned.csv") %>% readr::read_csv()
relative_neuropsych <- subset(neuropsych_df, neuropsych_df$group == "relative")
control_neuropsych <- subset(neuropsych_df, neuropsych_df$group == "control")
c9pos_neuropsych <- subset(neuropsych_df, c9_status_3 == "Positive")
c9neg_neuropsych <- subset(neuropsych_df, c9_status_3 == "Negative")

#### Run regression model for verbal fluency ####
model1 <- lm(fas_total_z ~ wasi_fsiq_2, data = neuropsych_df)
summary(model1)
lm.beta(model1)

model2 <- lm(fas_total_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
summary(model2)
lm.beta(model2)
anova(model1, model2)

plot(model2)

neuropsych_df$ Iowa_t

#### Run regression model for CWIT errors####
model1 <- lm(cwit_inhibition_errors_z ~ wasi_fsiq_2, data = neuropsych_df)
summary(model1)
lm.beta(model1)

model2 <- lm(cwit_inhibition_errors_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
summary(model2)
lm.beta(model2)
anova(model1, model2)
```



```
plot(model2)
```

```
#### Run model for digit span backwards ####
```

```
model1 <- lm(ds_backward_span_z ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(ds_backward_span_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

```
#### Run model for IGT trial 5 ####
```

```
model1 <- lm(net_5_t ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(net_5_t ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

```
#### Run model for BNT phonemic ####
```

```
model1 <- lm(bnt_spontaneous_semantic_phonetic_z ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(bnt_spontaneous_semantic_phonetic_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

```
#### Run model for ravlt immediate ####
```

```
model1 <- lm(ravlt_total_z ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(ravlt_total_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

```
#### Run model for LM delayed ####
```

```
model1 <- lm(lm2_total_ss ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(lm2_total_ss ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

```
#### Run model for RCFT delayed####
```

```
model1 <- lm(rcft_delayed_z ~ wasi_fsiq_2, data = neuropsych_df)
```

```
summary(model1)
```

```
lm.beta(model1)
```

```
model2 <- lm(rcft_delayed_z ~ wasi_fsiq_2 + group2, data = neuropsych_df)
```

```
summary(model2)
```

```
lm.beta(model2)
```

```
anova(model1, model2)
```

```
plot(model2)
```

R code for cluster analysis

```
library(tidyverse)
```

```
library(readxl)
```

```
library(here)
```

```
library(factoextra)
```

```
library(NbClust)
```

```
## load cleaned dataset ##
```

```
cluster_df <- here::here("cluster_analysis_2.csv") %>% readr::read_csv()
```

```
cluster_df_2 <- cluster_df[,8:28]
```

```
cluster_df_scale <- scale(cluster_df_2)
```

```
cluster_df_2 <- dist(cluster_df_scale)
```

```
fviz_nbclust(cluster_df_scale, kmeans, method = "silhouette") + labs(subtitle = "silhouette method")
```

```
##Suggests 2 clusters##
```

```
NbClust(cluster_df_scale, distance = "euclidean", min.nc=2, max.nc=15, method = "kmeans",  
index="all")
```

```
##Different plot
```

```
library(cluster)
```

```
set.seed(42)
```

```
km_res <- kmeans(cluster_df_scale, centers = 2, nstart = 100)
```

```
sil <- silhouette(km_res$cluster, dist(cluster_df_scale))
```

```
fviz_silhouette(sil)
```

```
##Different plot
```

```
fviz_cluster(km_res, na.omit(cluster_df_scale), ellipse.type = "norm")
```

```

##Recommends 2 as the best number of clusters

##Run cluster analysis

km.out <- kmeans(na.omit(cluster_df_scale), centers = 2, nstart = 100)

print(km.out)

km.clusters <- km.out$cluster


fviz_cluster(list(data = na.omit(cluster_df_scale), cluster = km.clusters), ellipse.type = "norm", repel =
TRUE,
              palette = "Set2", ggtheme = theme_minimal(), geom = "point")


##Add the clusters back to the dataset

cluster_df$cluster <- as.factor(km.out$cluster)


library(GGally)
library(plotly)


##Plot scores of different clusters ##

?write_csv

p <- ggparcoord(data = cluster_df, columns = c(8:28), groupColumn = "cluster", scale = "std") +
labs(x = "Candidate endophenotype", y = "value (in standard-deviation units)", title = "Clustering")

ggplotly(p)


write.csv(cluster_df, "cluster_nalysis_3.csv")


#compare clusters##

table(cluster_df$cluster, cluster_df$c9_status) #no difference
table(cluster_df$cluster, cluster_df$fam_hx) #no difference
table(cluster_df$cluster, cluster_df$gender) #no difference
table(cluster_df$cluster, cluster_df$handedness) #no difference
chisq.test(cluster_df$cluster, cluster_df$c9_status)
chisq.test(cluster_df$cluster, cluster_df$handedness)
chisq.test(cluster_df$cluster, cluster_df$gender)


t.test(age ~ cluster, cluster_df) #no difference

```

```

t.test(education ~ cluster, cluster_df) #no difference

## Compare scores on endophenotypes ##
t.test(wasi_fsiq_2 ~ cluster, cluster_df) #Sig difference
t.test(cwit_inhibition_errors_ss ~ cluster, cluster_df) #Sig difference
t.test(fas_total_z ~ cluster, cluster_df) #No difference
t.test(ds_backward_span_z ~ cluster, cluster_df) #No difference
t.test(rmet_total_z ~ cluster, cluster_df) #No difference
t.test(bnt_spontaneous_semantic_phonetic_z ~ cluster, cluster_df)
t.test(ravlt_total_z ~ cluster, cluster_df)
t.test(rcft_delayed_z ~ cluster, cluster_df)

wilcox.test(phq_total ~ cluster, cluster_df)
wilcox.test(gad_total ~ cluster, cluster_df)
wilcox.test(cape_total ~ cluster, cluster_df)
wilcox.test(ocir_total ~ cluster, cluster_df)
t.test(aq_ad ~ cluster, cluster_df)
t.test(das_total ~ cluster, cluster_df)
wilcox.test(asrs_total ~ cluster, cluster_df)
t.test(bis_total ~ cluster, cluster_df)
t.test(tipt_ext ~ cluster, cluster_df)
t.test(tipi_agree ~ cluster, cluster_df)
t.test(tipi_consc ~ cluster, cluster_df)
t.test(tipi_emot_stab ~ cluster, cluster_df)
t.test(tipi_open ~ cluster, cluster_df)

```

12.6. Appendix 6: Published works from this thesis

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2020; 21: 86–91



RESEARCH ARTICLE

Equivalency and practice effects of alternative versions of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS)

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Abstract

To examine the equivalency of ECAS versions A, B, and C in an Irish cohort, and to examine potential practice effects, 236 healthy controls were recruited through the Irish ALS control database. One hundred and seventy-six (176) controls completed ECAS version A, B, or C. Separately, 60 controls completed all three versions (A–B–C), consecutively, four months apart. TOST analysis found that ECAS A was equivalent to ECAS B and C. ECAS B and C were not statistically equivalent, however the difference between them was minimal. Participants showed improvement in ECAS performance over time, indicative of practice effects. Significant improvement was observed from time 1 to 2, but not from time 2 to 3. We propose Irish specific reliable change index (RCI) scores that take into consideration practice effects and measurement error. These thresholds will help quantify clinically meaningful cognitive decline in ALS patients, leading to improved quality of care.

Keywords: cognition, screen, ECAS, practice effects, equivalency

Introduction

The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) is a brief, yet sensitive screening tool in ALS (1). It has been translated into multiple languages (2–4) and has been validated against a full neuropsychological battery (5,6). In addition to detecting cognitive symptoms when they arise, it is equally important to track cognitive changes over time. Alternative versions of the ECAS (ECAS-B and ECAS-C) were developed specifically for this purpose (7).

In order to reliably measure clinically meaningful change from one time point to another, neuropsychologists will often utilize reliable change index (RCI) scores. RCI formulae generally take the form of a measure of true change as the numerator and the corresponding standard error as the denominator (8). An RCI approach is advantageous as it minimizes measurement error, can account for practice effects, and is easy to

interpret. RCI has been utilized to signify reliable change in a range of conditions such as schizophrenia, epilepsy, concussion, and dementia (9–12). Initial studies show that serial administration of alternative ECAS forms do not produce practice effects and RCI scores have been proposed for a Scottish population (13).

The purpose of this study is to examine if ECAS versions A, B, and C can be considered equivalent, and to generate normative data from an Irish population. This paper also investigates if serial administration of the ECAS produces practice effects in an Irish cohort and proposes RCI scores to help interpret cognitive change over time.

Method

Participants

Healthy Irish controls were recruited for two studies, one to assess the equivalency of ECAS

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Supplemental data for this article can be accessed [here](#).

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versions A, B, and C (study 1), and another to examine practice effects of serial administration (study 2). The first cohort of 176 participants completed either ECAS A, B, or C at one time-point. The second cohort of 60 participants underwent serial ECAS assessments, completing version A first, then B, then C. Exclusion criteria included a history of intellectual or learning disability, the presence of a mood disorder or psychiatric disorder, neurological conditions affecting cognition and/or a family history of ALS. All participants spoke English as their first language. Informed written consent was obtained from all participants.

Procedure

In study 1, participants ($n=176$) were administered either ECAS A, B, or C only. In study 2 ($n=60$), participants were administered ECAS A–B–C serially, approximately 4 months apart (4.2 months (median) between T1 and T2, inter quartile range = 4.0–4.6, and 4.3 months (median) between T2 and T3, inter quartile range = 4.0–4.8). Participants were tested in their own home, or if preferred, in a quiet room in Trinity Biomedical Science Institute, Dublin. Verbal fluency index (VFI) converted scores were generated using procedures outlined on the ECAS website: “<https://ecas.psy.ed.ac.uk/>” and are presented in Supplemental Table 1.

Data analysis

Statistical analysis was carried out using SPSS (Version 25, Armonk, NY) and R (Version 3.5.1, Vienna, Austria). Demographic information was compared using chi square tests and one-way analysis of variance (ANOVA). Shapiro–Wilks test, histograms, and normality plots were used to determine if data were parametric. Log transformations were performed for data which violated statistical assumptions. Where transformation failed to correct for violations, non-parametric tests were applied. To control for multiple comparisons, the Benjamini–Hochberg (14) procedure was implemented, using a false discovery rate of 5%. Power was calculated using G*power software (15).

Study 1. To investigate equivalency, one-way analyses of covariance (ANCOVA) was performed to compare ECAS total scores, composite scores, and sub-scores across groups. To detect a small effect size ($d=0.3$), with a power of 0.8, a minimum sample size of 111 was required. Age and education were added as covariates due to the significant relationships between these variables and ECAS performance (Supplemental Table 2). Kruskal–Wallis (K–W) tests were performed where data violated assumptions of parametric analysis. Two one sided t -tests (TOST) were performed to directly test the

equivalency between the ECAS groups. Unlike traditional tests, this procedure assumes that there is a difference between groups, specifying upper and lower limits that we consider meaningful (e.g. Cohens $d=-0.3$ to $+0.3$ to test a small effect). T -tests are then carried out on each limit. If the difference between the groups falls within these limits, we can conclude that they are equivalent (16).

In this TOST procedure, the pre-determined minimum meaningful difference between ECAS versions was a Cohens $d=-0.5$ to $+0.5$ as upper and lower limits respectively. An alpha level of 0.1 was applied (two tailed), giving a power of 0.8.

Study 2. To investigate practice effects, repeated measures ANOVA were carried out on ECAS total scores, composite scores, and sub-scores across each time point. In cases where Sphericity was violated, Greenhouse–Geisser, Huynh–Feldt, or lower bound corrections were applied. *Post hoc* sidak tests were used to compare sub-groups if a significant main effect was observed. To detect a small effect size ($d=0.15$) and achieve a power of 0.8, a minimum sample size of 31 was required. Test–retest reliability was assessed using intra-class correlation coefficients (ICC) with mean-rating absolute agreement two-way effects models. To provide clinically meaningful thresholds of cognitive decline, RCI scores were calculated. The Chelune method (17) of calculating RCI was applied. This approach takes into consideration the impact of practice effects and measurement error. An alternative standard error of difference (SE_{diff}), proposed by Iverson (18), was applied. This calculation of the standard difference is more appropriate as it takes into account variability in Time 2 scores. To calculate RCI scores, the following equation was utilized:

$$\Delta X = (\bar{X}_2 - \bar{X}_1) \pm 1.645 (SE_{diff})$$

where $SE_{diff} = \sqrt{SEM_1^2 + SEM_2^2}$, $SEM_1 = s^1 \sqrt{1 - r_{xx}}$, SEM is the standard error of measurement, s^1 is the standard deviation of the ECAS version being compared against (e.g. when comparing ECAS A and B, s^1 is the standard deviation of ECAS A), and r_{xx} is the test–retest correlation coefficient.

Results

Study 1: Equivalency of ECAS versions

One-way ANOVA and chi-square tests showed no significant difference between ECAS groups in terms of age, education, and gender (Table 1).

ANCOVA and K–W tests were carried out to compare mean ECAS performance across each ECAS version. No significant differences were observed between groups, except for the

Table 1. Mean (standard deviation) age, years of education, and gender frequency of participants who completed ECAS A, B, or C only.

	ECAS A (<i>n</i> = 70)	ECAS B (<i>n</i> = 52)	ECAS C (<i>n</i> = 54)	<i>F</i> / χ^2 (<i>df</i>)	<i>p</i> Value
Age	62.70 (8.65)	63.65 (10.27)	65.94 (11.09)	1.67	.19
Years of Education	15.53 (4.74)	16.79 (3.63)	15.55 (3.26)	1.77	.17
Gender	27F/43M	29F/23M	29F/25M	4.45	.11

Table 2. Mean (standard deviation), ANCOVA *F* value/K-W chi square, and *p* Value comparing ECAS A, B, and C.

	ECAS A	ECAS B	ECAS C	<i>F</i> / χ^2 (<i>df</i>)	<i>p</i> Value
ECAS total	114.16 (11.73)	115.85 (9.54)	113.07 (11.97)	0.34 (2, 171)	.71
ALS specific	85.07 (8.85)	86.27 (7.34)	83.85 (9.41)	0.49 (2, 171)	.62
Language	26.84 (1.66)	26.9 (1.76)	26.48 (1.79)	3.54 ^k (2, 176)	.17
Verbal fluency	19.71 (2.32)	19.69 (2.67)	18.93 (4.3)	0.90 (2, 171)	.41
Executive	38.51 (6.42)	39.67 (5.01)	38.44 (6.02)	0.47 (2, 171)	.63
ALS nonspecific	29.09 (4.62)	29.58 (3.87)	29.22 (3.85)	0.30 (2, 171)	.75
Memory	17.5 (4.3)	17.96 (3.67)	18.15 (3.31)	0.97 (2, 171)	.38
Visuospatial	11.59 (.83)	11.62 (.63)	11.07 (1.03)	15.08 ^k (2, 176)	.001*

^k – Assumptions of ANCOVA violated, Kruskal–Wallis test used.

*Statistically significant after controlling for multiple comparison.

Table 3. TOST procedure *t* value (*df*) and *p* values comparing ECAS A–B, A–C, and B–C.

	ECAS A–B TOST		ECAS A–C TOST		ECAS B–C TOST	
	<i>T</i> (<i>df</i>)	<i>p</i> Value	<i>T</i> (<i>df</i>)	<i>p</i> Value	<i>T</i> (<i>df</i>)	<i>p</i> Value
ECAS total	1.89 (119)	.03	–2.25 (113)	.01	–1.25 (100)	.11*
ALS specific	1.96 (119)	.03	–2.02 (111)	.02	–1.10 (100)	.14*
Language	2.53 (106)	.006	–1.60 (110)	.06	–1.36 (104)	.09
Verbal fluency	–2.66 (100)	.005	–1.46 (76)	.07	–1.49 (89)	.07
Executive	1.66 (120)	.05	–2.71 (117)	.004	–1.43 (102)	.08
ALS nonspecific	2.53 (106)	.02	–1.60 (109)	.005	–1.36 (104)	.02
Memory	2.13 (118)	.02	1.86 (122)	.03	2.29 (102)	.01
Visuospatial	2.56 (120)	.005	0.31 (100)	.62*	9.75 (88)	.77*

*Not statistically equivalent.

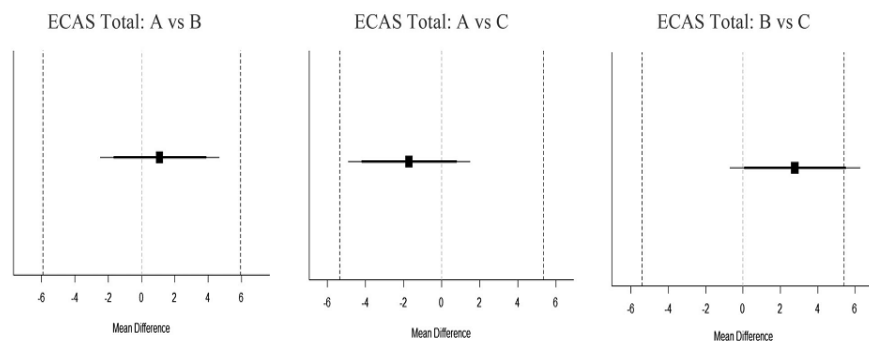


Figure 1. TOST results, displaying mean differences between ECAS A, B, and C total score.

visuospatial sub-test, where participants scored worse on version C, compared to ECAS A and B (Table 2).

In order to directly test the equivalency of ECAS versions the two one sided *t*-test (TOST) procedure was applied (Table 3). ECAS versions

A and B are equivalent on all ECAS domains. ECAS A and C were equivalent on all ECAS domains except visuospatial functioning. ECAS B and C were not equivalent for ECAS total, ALS specific scores or visuospatial functioning. Figure 1 illustrates the ECAS total TOST results.

Note that while ECAS B and C are not statistically equivalent, the difference between them is minimal.

Study 2: Practice effects of serial assessment

An independent cohort of participants completed serial ECAS assessment (see Table 4 for descriptives).

Repeated measures ANOVA were carried out to compare ECAS scores across each time point (Table 5). Statistically significant effects for time were observed for ECAS Total, ALS specific, and ALS nonspecific composite scores (Figure 2). Significant effects for time were also observed for verbal fluency and memory sub-scores. *Post hoc* sidak tests revealed that verbal fluency significantly improved from version A to B, while memory significantly improved from ECAS B to C.

Test-retest reliability: ICC were calculated for ECAS total, ALS specific, and ALS nonspecific scores (Supplemental Table 3). All ECAS versions

showed excellent test-retest reliability, with all coefficients higher than 0.7.

RCI scores were calculated using the Chelune method. This approach takes into account practice effects and measurement error. Based on these calculations we propose the following cutoffs as thresholds for determining clinically meaningful cognitive decline (Table 6).

Discussion

The results of this study suggest that alternative ECAS versions A, B, and C are comparable, but not strictly equivalent. A significant difference was observed on the visuospatial sub-task, in line with previous findings (13). TOST analysis found that ECAS A was equivalent to ECAS B and C, however, ECAS B was not equivalent to ECAS C. As with other studies, we recommend the use of population derived, version specific norms, and RCI scores when determining abnormal performance. Irish specific norms (Supplemental Tables 4 and 5) and RCI cutoffs are provided to aid this approach.

Table 4. Mean (standard deviation) and range of age, years of education, and gender frequency of participants who completed ECAS A–B–C serially.

	ECAS A–B–C (n = 60)	
	Mean (SD)	Range
Age	64 (7.36)	38–80
Years of education	15.42 (4.82)	8–40
Gender	39 M/21 F	

Table 6. Reliable change index (RCI) scores for ECAS A, B, and C.

	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

Table 5. Mean ECAS score, ANOVA *p* value, and *post hoc* sidak *p* value for serial ECAS assessment.

	ECAS A	ECAS B	ECAS C	<i>F</i> (<i>df</i>)	A–B mean difference	B–C mean difference
ECAS total	109.20 (16.09)	112.67 (14.96)	112.28 (16.45)	10.16* (2, 166)	−0.21	−0.14
ALS specific	80.97 (13.11)	83.75 (11.90)	82.43 (13.35)	7.49* (2, 118)	−0.21	0.08
Language	26.03 (3.20)	25.82 (2.97)	25.66 (3.25)	2.49 (2, 122)	–	–
Verbal fluency	18.52 (3.93)	19.90 (3.75)	19.21 (5.12)	11.40* (2, 120)	0.42*	−0.13
Executive	36.44 (7.39)	38.14 (6.86)	37.75 (6.90)	2.52 (2, 124)	–	–
ALS nonspecific	28.33 (4.62)	29 (4.19)	29.98 (7.43)	8.10* (2, 124)	0.12	0.16
Memory	16.81 (4.36)	17.78 (3.61)	18.51 (7.21)	11.57* (2, 124)	0.16	0.17*
Visuospatial	11.52 (.97)	11.22 (1.21)	11.21 (.98)	0.41 (2, 124)	–	–

*Statistically significant after controlling for multiple comparison.

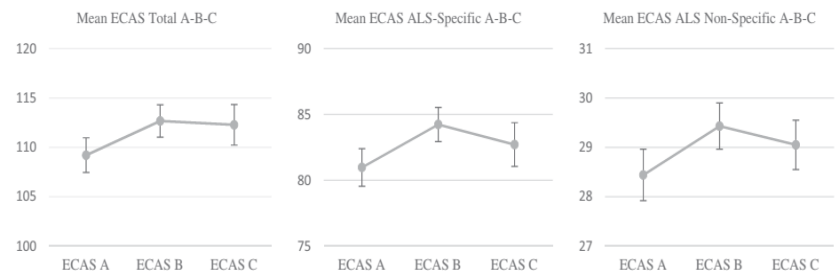


Figure 2. Mean ECAS total score on serial A–B–C assessment.

Contrary to previous research (13), this study found evidence of practice effects on the ECAS over time. Participants significantly improved from time one to time two, particularly on the verbal fluency task. Participants did not improve on ECAS total from time 2 to time 3, however, there was significant improvement on the memory sub-task. Practice effects in neuropsychological batteries are most common on novel tasks where participants can learn advantageous test strategies (19). This may account for the slight improvement in verbal fluency. Previous studies have also shown that the largest improvements are usually from time 1 to time 2, and then reduced thereafter (20). Improvement may be due to participants becoming increasingly comfortable with testing conditions or through improved task learning strategies (21). While alternative forms do not completely remove the presence of practice effects, they do greatly reduce them. Repeated administration of ECAS A (A-A-A) produces much larger practice effects than those found from serial assessment (A-B-C) (22). This study retested individuals in intervals of 4 months, in line with previous research (13,22). Longer intervals may reduce the presence of practice effects however further study is required. Future research is also needed to examine a broader range of ages and the effects of alternative ECAS sequences, for example, C-A-B, B-C-A, etc.

In order to provide clinicians with a practical means of identifying cognitive change over time, while accounting for potential practice effects, we present Irish specific RCI cutoffs. Decline or improvement greater than these thresholds can be considered indicative of clinically meaningful change. For example, decline greater than 10 from ECAS A to B, or greater than 7 from ECAS B to C on ECAS total, can be considered clinically meaningful and warrant further investigation. These thresholds compliment previously proposed cutoffs by Crockford et al. (13), who proposed a decline of 9 on ECAS total as an indicator of clinically meaningful change in a Scottish cohort. The publication of Irish specific cutoffs and normative data add to the utility of the ECAS as a screening measure across various countries, allowing reliable comparison between patients from different countries.

While RCI scores will give greater clarity to those applying the ECAS, it is essential to bear in mind the limitations of this approach. As with any unidimensional metric of abnormality, RCI thresholds fail to account for individual differences. Clinicians must be vigilant that age, education, baseline performance, and differential practice effects are unique to each individual (23). This study is limited by the small range of age and education of participants and by the fact that anxiety

and depressive were not measured directly. Previous research has shown the ECAS to be highly sensitive and specific. However, it is important to acknowledge its intended use is as a screening tool and, when possible, should be followed up by a full neuropsychological assessment by a trained neuropsychologist. Given the contrasting findings between this study, and previous literature, further research is needed to clarify the psychometric properties of alternative ECAS versions, and the extent to which practice effects occur. In doing so, the ECAS will become an increasingly useful tool in understanding the nature of cognitive and behavioral deficits in ALS, informing clinicians in everyday care, and providing a useful metric for clinical trials.

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Data availability

The dataset analyzed during the current study are available from Mr. Mark Heverin, Research Manager, upon reasonable request.

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Original research

Cognitive reserve in amyotrophic lateral sclerosis (ALS): a population-based longitudinal study

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ABSTRACT

Background Amyotrophic lateral sclerosis (ALS) is often associated with cognitive and/or behavioural impairment. Cognitive reserve (CR) may play a protective role in offsetting cognitive impairment. This study examined the relationship between CR and longitudinal change in cognition in an Irish ALS cohort.

Methods Longitudinal neuropsychological assessment was carried out on 189 patients over 16 months using the Edinburgh cognitive and behavioural ALS screen (ECAS) and an additional battery of neuropsychological tests. CR was measured by combining education, occupation and physical activity data. Joint longitudinal and time-to-event models were fitted to investigate the associations between CR, performance at baseline and decline over time while controlling for non-random drop-out.

Results CR was a significant predictor of baseline neuropsychological performance, with high CR patients performing better than those with medium or low CR. Better cognitive performance in high CR individuals was maintained longitudinally for ECAS, social cognition, executive functioning and confrontational naming. Patients displayed little cognitive decline over the course of the study, despite controlling for non-random drop-out.

Conclusions These findings suggest that CR plays a role in the presentation of cognitive impairment at diagnosis but is not protective against cognitive decline. However, further research is needed to examine the interaction between CR and other objective correlates of cognitive impairment in ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of the upper and lower motor neurons that results in a relentless paralysis and death.¹ Fifteen percent of patients with ALS meet criteria for frontotemporal dementia (FTD), while 30% of non-demented patients will have cognitive impairment.² These changes have a detrimental effect on patient adherence to treatment, their survival and are associated with greater caregiver burden.^{3–5}

It remains unclear as to the point at which cognitive impairment emerges and the extent to which cognition declines in ALS. Recent studies suggest that cognitive decline coincides with disease stage (using both Kings and MiTos staging

systems),^{6,7} although these data are based on cross-sectional design. Longitudinal studies have largely found that cognitive impairment emerges early in the disease. Those who are impaired at diagnosis subsequently decline over time while those who are unimpaired at diagnosis appear to remain cognitively intact throughout their illness.^{8–11} These studies are often limited by their high attrition rates, biasing estimates of true decline due to drop-out of those with faster disease progression. Joint longitudinal and time to event models (joint models) have gained popularity for their utility in handling data missing-not-at-random and improving statistical power.¹² By applying joint modelling to longitudinal neuropsychological data in ALS, we can get a better estimate of the true rate of cognitive decline.

A potential mediator of cognitive change, which may account for some of the varied presentations in ALS, is cognitive reserve (CR). Theories of CR stipulate that neural enrichment, through education, occupation and physical activity increases an individuals' neural resources (eg, greater grey matter volume or white matter tract integrity).¹³ As the disease spreads across networks, individuals recruit reserve neural resources to deal with cognitive demands, a process known as compensation. Educational attainment, occupational complexity and leisure activity are often used as proxy measures of CR, either in isolation or combined to form a latent construct.

There is indirect evidence that CR plays a protective role in ALS. ALS patients with comorbid FTD are older, have lower educational attainment and poorer survival.¹⁴ Executive functioning and verbal memory are protected by CR in a range of other neurodegenerative conditions.^{14–18}

Here, we have examined the relationship between CR and longitudinal neuropsychological performance in ALS. We hypothesise that higher CR is associated with improved cognitive performance at baseline and with a reduced slope of decline over time. We have applied a multiple variable model, using the shared variance of several variables, to create a 'latent' measure of CR which can offer a less biased measure than using an individual proxy in isolation.¹⁹

METHODS

Participants

One hundred and eighty-nine patients with ALS were recruited for this longitudinal,



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Cognition

population-based research study. Participants were recruited as part of ongoing longitudinal studies of neuropsychological deficits in an Irish ALS cohort from 2012–2019.⁸ Participants were assessed within the first year of their diagnosis and reassessed three further times, 4 months apart. Assessments took place in a quiet room in Beaumont Hospital, Dublin, Ireland or in the participants home. Exclusion criteria included: (1) history of an intellectual or learning disability, (2) history of a comorbid neurological, psychiatric or medical condition affecting cognition, (3) alcohol dependence syndrome, (4) if the person was a non-native English speaker or (5) a comorbid diagnosis of FTD. Occupational history, education and physical activity data were derived from Irish EuroMOTOR project data, a study of environmental risk factors of ALS.²⁰ Clinical data were accessed through the Irish ALS register.

Materials

CR score was calculated by combining traditional proxies of CR, namely: education, occupation and physical activity. Education score was the total years an individual spent in full time education. Occupation was coded according to complexity using the International Standard Classification of Occupations-88, inverted so that jobs with increasing complexity had higher scores, and then categorised into nine categories ranging from 1 (elementary occupations) to 9 (legislators, senior officials and managers). Occupation score was the sum of years a person spent in each occupation multiplied by its category. This approach matches how the CR index questionnaire was developed,²¹ capturing the time an individual spent in a job as well as the cognitive complexity of that job. Physical activity score was the total number of years a person reported as engaging in each sport/exercise. Each CR subdomain was converted to a z-score using the sample mean and SD and then averaged to give a CR total score.

In the absence of direct biomarkers of the underlying spread of pathological changes, disease progression was approximated using time from disease onset to neuropsychological assessment,¹⁹ as described in previous studies.^{15 22 23}

The Edinburgh cognitive and behavioural ALS screen (ECAS) is a sensitive screening tool of cognition in ALS.²⁴ It measures three ALS-specific functions, language, verbal fluency and executive functioning, and 2 ALS non-specific functions, memory and visuospatial functioning. To examine cognition over time, while limiting the influence of practice effects, alternative ECAS versions were used, that is, ECAS A-B-C.²⁵

Participants were also administered the FAS verbal fluency test, the Boston Naming Test (BNT), the Reading the Mind in the Eyes Test (RMET), the Colour-Word Interference Test (CWIT), the Logical Memory test from the Wechsler Memory Scale-III and the Rey Auditory Verbal Learning Test (RAVLT). These provided accurate measurements of executive functioning,²⁶ verbal fluency,²⁷ language,²⁸ memory,^{29 30} and social cognition.³¹ To limit the influence of motor disability, Verbal Fluency Index was calculated on verbal fluency tasks and total errors was used instead of completion time on the CWIT, as described in previous studies.³² Cognitive performance raw scores were converted to z-scores using test manuals and published age, IQ and gender matched healthy control normative data.^{26–31} A summary of each neuropsychological test, the cognitive domain it measures, and a brief description of the task can be found in table 1.

Statistical analysis

Linear mixed effects models were used to examine the association between CR and neuropsychological performance over the course of 16 months. Linear mixed effects models are a robust means of dealing with missing data, a particularly relevant issue with such a rapidly progressing population with high attrition. We applied additional methods to cater for non-linear data (see online supplemental material). In these models the intercept and slope were fitted as random effects, allowing them to vary for each individual. Each mixed model included fixed effect terms for time, age, CR score and an interaction between CR and time. The fixed effect term for CR represents the association between baseline neuropsychological performance and CR. The time by CR interaction term represents the association between the slope of change over time and CR. A Cox survival model was

Table 1 Neuropsychological battery

Cognitive domain	Test name	Description
Cognitive screening	ECAS total	Composite score of ALS specific and ALS non-specific scores.
	ECAS ALS specific	Includes short tests of language, verbal fluency and executive functioning.
	ECAS ALS non-specific	Includes short tests of visuospatial functioning and memory.
Verbal fluency	FAS (unrestricted) fluency*	Generate as many words as possible beginning with F, A and S, 1 min for each letter.
	Restricted fluency*	Generate as many words as possible beginning with C, with only four letters. Given 1 min.
Language	Boston naming test	Name 30 items.
Social cognition	Reading the mind in the eyes test	Infer the emotional state from 30 faces, given four options on each item.
Memory	Logical memory (LM) WMS-III†—immediate recall	Recall two short stories, immediate recall and delayed recall after 20 min
	LM WMS-III—delayed recall	
	List recall RAVLT† immediate recall	Recall a list of 15 words five times, delayed recall after 20 min
Executive functioning	List recall RAVLT delayed recall	
	Colour-Word Interference Test (CWIT) inhibition§	Must identify the ink colour of incongruent word-colour pairs.
	CWIT switching/inhibition§	Must switch between two rules; identify the ink colour or name the word of incongruent word-colour pairs

*Verbal fluency index was calculated to account for motor impairment.

†Wechsler Memory Scale, Third version.

‡Rey Auditory Verbal Learning Task.

§Total errors used instead of completion time to control for bulbar impairment.

ALS, amyotrophic lateral sclerosis; ECAS, Edinburgh cognitive and behavioural ALS screen; RAVLT, Rey Auditory Verbal Learning Test; WMS, Wechsler Memory Scale.

Table 2 Demographic characteristics, including age, education, sex, site of onset, time from onset to baseline, familial/sporadic family history and C9orf72 status at each time point

Participant characteristics	Time 1 (n=189)	Time 2 (n=117)	Time 3 (n=80)	Time 4 (n=49)
Age, mean years (SD)	63.59 (11.42)	61.64 (12.09)	60.52 (12.64)	58.94 (12.57)
Education, mean years (SD)	13.8 (3.82)	13.97 (3.41)	14.28 (3.36)	13.94 (2.93)
Sex, n				
Male	119	77	54	34
Female	70	40	27	15
Site of onset, n				
Bulbar	44	24	12	4
Spinal	145	93	69	45
Time from onset to assessment, mean months (SD)	19.48 (13.29)	25.4 (15.1)	30.11 (14.74)	34.53 (15.52)
ALSFRS-R, mean (SD)	36.5 (7.6)	33.43 (7.84)	32.34 (8.29)	31.4 (8.63)
Delta ALSFRS-R, change in ALSFRS-R per month	–	0.52	0.23	0.21
Familial/sporadic, n				
Familial	31	23	17	13
Sporadic	158	94	63	36
C9orf72 status, n				
Positive	15	11	6	4
Negative	174	106	75	45
El escorial diagnostic status, n				
Definite	92	50	33	23
Probable	59	44	29	14
Possible	38	23	18	12
Strong criteria status, n				
ALSn	140	89	67	43
ALSci	33	19	9	5
ALSbi	6	2	1	0
ALSchi	10	7	3	1

ALS, amyotrophic lateral sclerosis; ALSbi, ALS with behavioural impairment; ALSchi, ALS with cognitive and behavioural impairment; ALSci, ALS with cognitive impairment; ALSFRS-R, ALS Functional Rating Scale -Revised; ALSn, ALS with normal cognition and behaviour.

constructed with survival as the outcome variable, and known risk factors for shorter survival as predictors, namely age,³³ diagnostic delay,³⁴ site of onset³⁵ and C9orf72 repeat expansion status (see [table 2](#)).³⁶

The longitudinal mixed model and Cox survival models were then used to create joint longitudinal and time-to-event models (joint models), using the R package JMBayes.³⁷ This analysis enabled the model to control for non-random drop-out in our longitudinal data, resulting in less biased effect estimates. Joint models also provide a measurement of the association between each cognitive measure and death.³⁸ Analyses were carried out using R statistical software V3.6.3.³⁹

Informed written consent was given by all participants.

RESULTS

Demographic information

Participant demographic information at each time point is provided in [table 2](#). Participants first assessment occurred on average 19 months after their first symptoms. Attrition rate was 79% over the course of the study, representative of the rapidly progressive nature of the disease.

CR as a predictor of cognition at baseline

CR score was a significant predictor of baseline performance on the ECAS (including ALS-specific and non-specific subscores), RMET, CWIT (inhibition and switching scores), BNT and logical memory (immediate and delayed recall). Higher CR was positively associated with higher test performance. However, CR was not a significant predictor of baseline verbal fluency or RAVLT (total or delayed) score (see [table 3](#)). Full model summaries are provided in online supplemental table 1.

CR as a predictor of longitudinal cognition

Longitudinal neuropsychological scores, adjusted for age, site of onset, diagnostic delay and C9orf72 status through joint modelling, are displayed in [figure 1](#).

Individuals were divided into high (CR z-score greater than 1), medium (CR z-score between –1 and 1) and low (CR z-score < –1) CR groups to illustrate the effect of CR over time. For ECAS, RMET, CWIT and BNT, high CR individuals displayed greater performance than medium and low CR groups over time. For logical memory, high medium and low CR regressed towards the mean over time, with high CR groups declining and low CR groups improving.

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Table 3 Cognitive reserve fixed effect for each neuropsychological test from joint model summary

Outcome	β (SE)	95% CI	P value
ECAS total	0.37 (0.003)	0.11 to 0.64	0.003
ECAS ALS specific	0.40 (0.002)	0.21 to 0.59	<0.001
ECAS ALS non-specific	0.26 (0.003)	0.06 to 0.44	0.01
Reading the mind in the eyes	0.66 (0.005)	0.26 to 1.04	0.002
Verbal fluency (unrestricted)	0.49 (0.007)	-0.15 to 1.16	0.13
Verbal fluency (restricted)	0.61 (0.02)	-0.73 to 1.99	0.39
CWIT inhibition	0.87 (0.008)	0.27 to 1.49	0.009
CWIT switching	0.63 (0.09)	0.17 to 1.07	0.008
Boston naming task	0.55 (0.005)	0.13 to 0.98	0.01
RAVLT total	0.29 (0.004)	-0.02 to 0.59	0.07
RAVLT delayed	0.27 (0.004)	-0.15 to 0.58	0.25
Logical memory immediate	0.38 (0.004)	0.02 to 0.72	0.04
Logical memory delayed	0.56 (0.005)	0.11 to 0.99	0.03

ALS, amyotrophic lateral sclerosis; CWIT, Colour-Word Interference Test; ECAS, Edinburgh cognitive and behavioural ALS screen; RAVLT, Rey Auditory Verbal Learning Test.

Survival risk factors

Joint models rely on the construction of Cox survival models to control for non-random drop-out. Controlling for the effects of age, diagnostic delay, site of onset and C9orf72 repeat expansion status, lower ECAS ALS-specific and RAVLT total score were associated with shorter survival. Conversely, higher logical memory immediate score was associated with shorter survival. Table 4 displays the association between each longitudinal outcome and survival after joint modelling using JMBayes, specified with the current value association structure.

DISCUSSION

This study found that CR is associated with baseline differences in neuropsychological performance in a population-based cohort. These differences were most notable for ECAS, social cognition, executive functioning and confrontational naming, where higher performance in high CR individuals was maintained 16 months from baseline. Verbal fluency was not associated with higher CR despite the fact that fluency is often the most sensitive test for cognitive impairment in ALS. These findings may suggest that CR does not protect against fluency deficits, or that the disease has progressed to the stage where it has overcome any protective effect for this function.

For episodic memory (ie, logical memory tasks), high CR individuals performed better at baseline but then declined to a greater extent than low CR groups over time. This may suggest that CR plays a differential role in protecting functions commonly affected by ALS, namely executive functioning, language and social cognition, but is less influential on less implicated functions, such as memory.

ECAS ALS-specific deficits were associated with shorter survival, which is consistent with previous studies,³ as was RAVLT total score. Higher logical memory immediate score was associated with shorter survival. While executive impairment is a known risk factor for shorter survival in ALS, the role of memory is less established. Similar findings have been observed in Alzheimer's research, where individuals with higher CR perform better on memory tasks in the early disease stage, but then suffer from a more rapid disease decline.⁴⁰

These findings in an ALS cohort are similar to those of other neurodegenerative diseases. In Parkinson's disease high CR patients perform better on attention, executive functioning and

visuospatial tasks, and show a slightly reduced rate of decline.¹⁵ In Huntington's disease, higher CR is associated with better cognition in patients and in prodromal gene carriers over time.¹⁶ In Alzheimer's disease, there is evidence of a different pattern. Higher CR is associated with better performance at baseline, but this is followed by a sharper rate of decline once symptoms emerge.⁴⁰ This suggests that CR may operate by delaying the onset of clinical symptoms rather than reducing the overall rate of decline.

The factors that contribute to preserved cognition in high CR individuals remain unclear, but could relate to overall network integrity, mediated by in part by preserved grey matter volume.¹³ In this case, the high CR individual may be better able to compensate for disease mediated neuronal injury and network disruption.

The results of this study support previous longitudinal studies in ALS that those with normal cognition remain relatively stable over time, at least following diagnosis.^{8–10} The utilisation of joint models to control for non-random drop-out, suggests that the lack of decline is not solely attributable to the drop-out of highly impaired patients.

Limitations

Our analysis of cognition was limited to 16 months follow-up; therefore, we have not characterised the rate of decline over the longer-term disease course. This study is also limited by the lack of a control group which would have given an indication of practice effects and the extent to which scores regress towards the mean. Future studies should compare the role of CR over a longer period, and relative to healthy controls and similar patient groups.

Disease progression was approximated using time since symptom onset to the point of neuropsychological assessment. This assumes a linear relationship between time and disease progression, which is not often the case. Furthermore, this study is limited by the lack of neuropathological and neuroimaging data. Further work will be required to characterise the relationship between CR and the underlying neuropathological, neuroimaging and neuroelectric changes associated with cognitive impairment in ALS. Specifically, studies may consider examining if CR moderates the relationship between DT-MRI (Diffusion Tensor Magnetic Resonance Imaging) abnormalities in the corpus callosum and frontotemporal tracts and neuropsychological outcomes.⁴¹ Similarly, studies could explore if CR mediates the association between ¹⁸F-FDG-PET (Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography) measured hypometabolism in the frontal cortex and cognitive impairment.^{42–44}

Conclusion

Our findings show that higher CR is associated with better neuropsychological performance, particularly in domains associated with ALS, where the differential between high and low CR was maintained over time. However, given the limitations of the study, future research is required to explore the relationship between CR and the underlying neuroimaging, neuroelectric and neuropathological signatures of impairment in ALS.

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Contributors EC, OH and NP designed and conceptualised the study. EC, JR, MP-G, TB, ME, PB, MH and AV played a major role in the acquisition of data. EC and JR analysed and interpreted the data. EC drafted the manuscript for intellectual

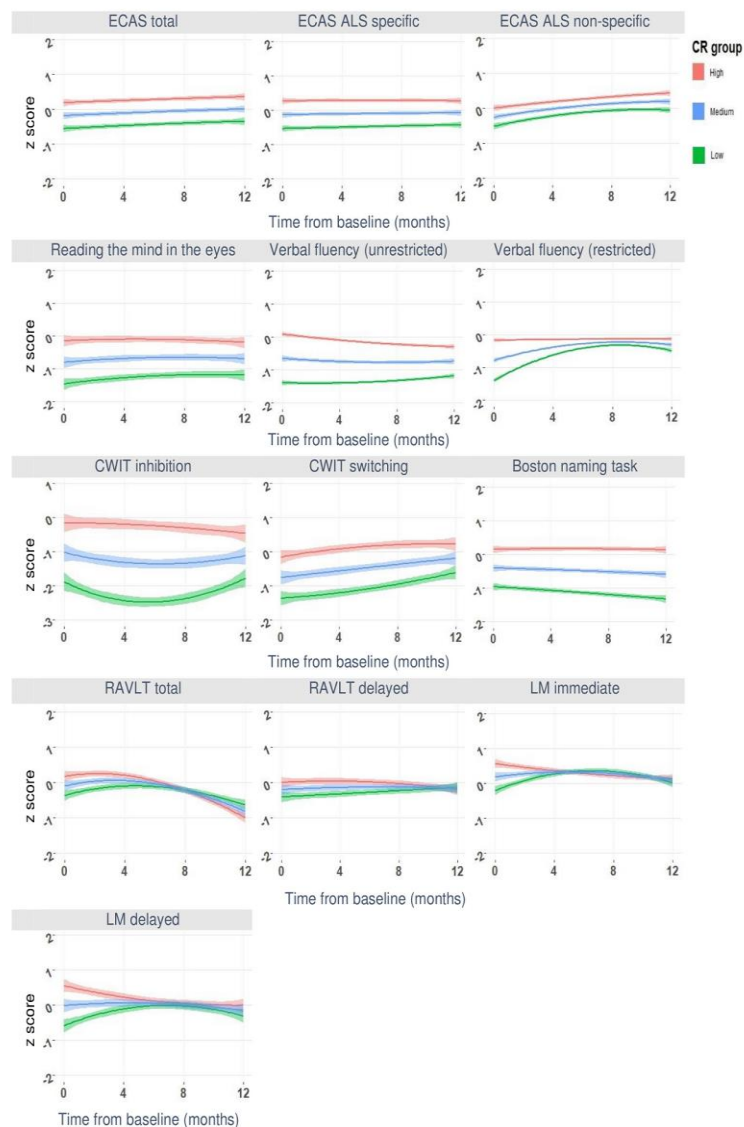


Figure 1 High CR defined as having a CR Z score >1 ; medium CR defined as having a CR Z score between $+1$ and -1 ; low CR defined as having a CR Z score <-1 . ALS, amyotrophic lateral sclerosis; CR, cognitive reserve; CWIT, Colour-Word Interference Test; ECAS, Edinburgh cognitive and behavioural ALS screen; LM, logical memory; RAVLT, Rey Auditory Verbal Learning Test.

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Competing interests NP serves as Associate Editor of the International Journal of Neuroscience and has received speaker honoraria from Novartis. OH has received speaking honoraria from Janssen Cilag, Biogen Idec, Sanofi Aventis, Novartis and

MerckSerono. She has been a member of advisory panels for Biogen Idec, Allergan, Ono Pharmaceuticals, Novartis, Cytokinetics and Sanofi Aventis. She serves as editor-in-chief of the journal Amyotrophic Lateral Sclerosis and Frontotemporal Dementia.

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Table 4 Estimate of HR for neuropsychological scores after joint longitudinal and time to event modelling

Variable	HR (95% CI)
ECAS total	0.88 (0.73 to 1.06)
ECAS ALS Specific	0.65 (0.55 to 0.77)*
ECAS ALS-non-specific	0.99 (0.84 to 1.16)
Reading the mind in the eyes	1.17 (0.89 to 1.64)
Verbal fluency (unrestricted)	0.93 (0.85 to 1.02)
Verbal fluency (restricted)	1.04 (0.99 to 1.25)
CWIT inhibition	1.09 (0.89 to 1.32)
CWIT switching	0.99 (0.97 to 1.01)
Boston naming test	0.99 (0.86 to 1.17)
RAVLT total	0.99 (0.92 to 0.99)*
RAVLT delayed	0.81 (0.55 to 1.02)
Logical Memory immediate	1.80 (1.17 to 3.07)*
Logical memory delayed	1.39 (0.57 to 2.68)

*P<0.001.

ALS, amyotrophic lateral sclerosis; CWIT, Colour-Word Interference Test; ECAS, Edinburgh cognitive and behavioural ALS screen; RAVLT, Rey Auditory Verbal Learning Test.

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Supplementary material

Supplementary table 1. Longitudinal sub-model summary of neuropsychological performance over time.

Outcome	Predictor	β	95% CI	p	Outcome	Predictor	β	95% CI	p
ECAS total	Time (spline 1)	0.45	-0.04 – 0.94	.07	ECAS ALS Specific	Time (spline 1)	0.12	-0.58 – 0.80	.69
	Time (spline 2)	0.30	-0.24 – 0.81	.27		Time (spline 2)	0.05	-1.06 – 1.11	.89
	Age	-0.03	-0.04 – -0.02	<.001		Age	-0.03	-0.03 – -0.01	<.001
	Cognitive reserve	0.37	0.11 – 0.64	.003		Cognitive reserve	0.40	0.21 – 0.59	<.001
	CR x time (spline 1)	-0.03	-0.73 – 0.68	0.92		CR x time (spline 1)	-0.15	-1.08 – -0.81	.74
	CR x time (spline 2)	0.04	-0.75 – 0.82	0.94		CR x time (spline 2)	-0.17	-1.65 – 1.35	.81
ECAS ALS	Time (spline 1)	0.34	-0.19 – 0.90	.21	RMET	Time (spline 1)	0.25	-0.49 – 0.96	.51
Non-Specific	Time (spline 2)	-1.98	-2.07 – -1.86	<.001		Time (spline 2)	-0.07	-0.35 – 0.23	.62
	Age	-0.02	-0.03 – -0.01	<.001		Age	-0.05	-0.07 – -0.03	<.001
	Cognitive reserve	0.26	0.06 – 0.45	.01		Cognitive reserve	0.66	0.26 – 1.04	.002
	CR x time (spline 1)	0.73	-0.05 – 1.51	.07		CR x time (spline 1)	-0.25	-1.33 – 0.81	.63
	CR x time (spline 2)	2.15	1.99 – 2.33	<.001		CR x time (spline 2)	-0.15	-0.59 – 0.28	.43
Verbal	Time (spline 1)	-0.20	-1.55 – 1.13	.77	Verbal (Restricted)	Time (spline 1)	0.95	0.04 – 1.80	.05
Fluency (Unrestricted)	Time (spline 2)	0.07	-0.52 – 0.66	.82		Time (spline 2)	-0.05	-0.76 – 0.61	.89
	Age	-0.03	-0.05 – -0.01	.005		Age	-0.02	-0.05 – 0.01	.32
	Cognitive reserve	0.49	-0.15 – 1.16	.13		Cognitive reserve	0.61	-0.73 – 1.99	.39
	CR x time (spline 1)	-0.27	-2.22 – 1.69	.78		CR x time (spline 1)	-0.89	-2.19 – 0.34	.17
	CR x time (spline 2)	-0.26	-1.08 – 0.58	.54		CR x time (spline 2)	0.07	-0.94 – 1.10	.89
CWIT	Time (spline 1)	-0.48	-0.87 – -0.07	.03	BNT	Time (spline 1)	-0.25	-0.95 – 0.45	.48
Inhibition	Time (spline 2)	0.44	-0.20 – 1.12	.16		Time (spline 2)	-0.26	-1.08 – 0.53	.51
	Age	-0.08	-0.11 – -0.05	<.001		Age	-0.03	-0.05 – -0.01	.001
	Cognitive reserve	0.87	0.27 – 1.49	.01		Cognitive reserve	0.55	0.13 – 0.98	.01
	CR x time (spline 1)	0.14	-0.40 – 0.65	.58		CR x time (spline 1)	0.26	-0.75 – 1.26	.64
	CR x time (spline 2)	-0.92	-1.82 – -0.05	.04		CR x time (spline 2)	0.20	-0.98 – 1.34	.72
CWIT	Time	-1.04	4.00 – 1.93	.50	RAVLT total *	Time	-1.49	-9.86 – 6.63	.72
Switching *	Age	-0.06	-0.09 – -0.04	<.001		Age	-0.04	-0.06 – -0.02	<.001
	Cognitive reserve	0.63	0.17 – 1.07	.008		Cognitive reserve	0.29	-0.02 – 0.59	.07
	CR x time	0.56	-3.12 – 4.26	.78		CR x time	-4.49	-14.99 – 6.55	.41

RAVLT delayed	Time (spline 1)	0.11	-0.23 – 0.44	.50	LM Immediate	Time (spline 1)	0.05	-0.34 – 0.49	.84
	Time (spline 2)	-0.08	-1.22 – 1.05	.91		Time (spline 2)	-0.57	-0.96 – -0.14	.009
	Age	-0.04	-0.06 – -0.02	<.001		Age	-0.04	-0.06 – -0.02	.002
	Cognitive reserve	0.21	-0.15 – 0.58	.25		Cognitive reserve	0.38	0.03 – 0.72	.04
	CR x time (spline 1)	-0.27	-0.72 – 0.17	.22		CR x time (spline 1)	-0.73	-1.32 – -0.17	.02
	CR x time (spline 2)	-0.52	-1.84 – 1.01	.57		CR x time (spline 2)	0.20	-0.30 – 0.72	.44
LM Delayed	Time (spline 1)	0.11	-0.23 – 0.44	.50					
	Time (spline 2)	-0.08	-1.22 – 1.05	.91					
	Age	-0.04	-0.06 – -0.02	<.001					
	Cognitive reserve	0.21	-0.15 – 0.58	.25					
	CR x time (spline 1)	-0.27	-0.72 – 0.17	.22					
	CR x time (spline 2)	-0.52	-1.84 – 1.01	.57					

* Splines not added as they did not improve the fit of the model

Model Specification

Joint longitudinal and time-to-event models are composed of two sub-models, a Cox survival model and a longitudinal mixed effects model. The dependence between the two sub-models is captured through the association structure. In this case a current value association structure was chosen, which assumes the log hazard function of the event at time t is linearly associated with the longitudinal sub-model predictor at time t .

Cox survival model

Known risk factors for shorter survival in ALS were included in the Cox model, i.e. age, diagnostic delay, site of onset and C9orf72 positive status. The Cox survival model is:

$$h_i(t) = h^0(t) \times \exp(\alpha^1 \times m_i(t) + b^1 x_i^1 + b^2 x_i^2 + b^3 x_i^3 + b^4 x_i^4)$$

or

$$h_i(t) = h^0(t) \times \exp(\alpha^1 m_i(t) + b^1(\text{age}_i) + b^2(\text{diagnostic delay}_i) + b^3(\text{site of onset}_i) + b^4(\text{C9orf72}_i))$$

where: (t) is survival time, h(t) is the hazard function determined by covariates x^1, x^2, x^3, x^4 .

h^0 is the baseline hazard where x^1, x^2, x^3 and x^4 equal 0. α^1 is the association parameter, representing the strength of association between the longitudinal outcome measure (i.e. cognitive score) and the time-to-event outcome. $m_i(t)$ is the expectant value predicted by the longitudinal model for a given individual at time = t.

x^1 = age, x^2 = diagnostic delay, x^3 = site of onset, x^4 = C9orf72 status. The coefficients b^1, b^2, b^3, b^4 measure the effect size of each covariate.

Longitudinal Mixed Effects Model

Longitudinal mixed effects models were fit for each outcome measure. Time was defined by months since baseline assessment. Natural cubic splines with two degrees of freedom were added to cater for non-linear trends over time. Age, CR and a time by CR interaction were included as fixed effects. Random intercepts and random slopes for time were included. The mixed effects equation for each outcome measure is:

$$m_i(t) = \beta^0 + \beta^1 x_i^1 + \beta^2 x_i^2 + \beta^3 x_i^3 + \beta^4 x_i^4$$

or

$$m_i(t) = \beta^0 + \beta^1 (\text{time}_i) + \beta^2 (\text{age}_i) + \beta^3 (\text{CR}_i) + \beta^4 (\text{CR} * \text{time}_i) + \epsilon_i$$

where

$$y_i(t_{ij}) = m_i(t_{ij}) + \epsilon_{ij}$$

and $y_i(t_{ij})$ represents the j th observational value for patient i at time t_{ij}

and $x^1 = \text{time}$, $x^2 = \text{age}$, $x^3 = \text{CR}$, $x^4 = \text{CR by time interaction}$, $\varepsilon = \text{random error}$. Models were evaluated comparing Akaike's information criterion (AIC), Bayesian information criterion (BIC) and log likelihood ratio test.

13. Supplementary information

Supplemental table 1. Verbal fluency conversion score table for ECAS versions B and C.

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

Supplemental table 2. Correlation between age, education and ECAS performance.

		Age		Education	
		Pearson r/ Spearman rho	p	Pearson r/ Spearman rho	p
ECAS A	Total	-.15	.23	.06	.65
	ALS Specific	-.12	.31	.07	.55
	ALS Non-Specific	-.13	.28	.003	.98
ECAS B	Total	-.02 ^s	.91	.27 ^s	.05
	ALS Specific	.06	.66	.29	.04
	ALS Non-Specific	-.33	.02	.27	.05
ECAS C	Total	-.46	.001	.32	.02
	ALS Specific	-.42	.001	.32	.02
	ALS Non-Specific	-.39	.004	.21	.13

s – Data non-parametric, Spearman's rho used

Supplemental table 3. Intraclass correlation coefficient and standard error of difference of serial ECAS assessment (A-B-C).

	<i>ECAS</i>			<i>ECAS</i>	<i>ECAS</i>	<i>ECAS</i>
				<i>A - B</i>	<i>B - C</i>	<i>A - C</i>
	r_{xx1}	r_{xx2}	r_{xx3}	SE_{diff}	SE_{diff}	SE_{diff}
ALS Specific	.86	.93	.92	6.63	4.02	4.57
ALS Non-Specific	.76	.85	.81	3.05	2.20	2.62
ECAS Total	.87	.95	.93	7.90	4.29	5.31

Note. r_{xx1} is the intraclass correlation coefficient of ECAS-A-B, r_{xx2} is the intraclass correlation coefficient of ECAS-B-C, r_{xx3} is the intraclass correlation coefficient of ECAS-A-C, SE_{diff} is the standard error of the difference.

Supplemental table 4. Abnormality cut-offs for ECAS Version B, based on healthy Irish controls who completed ECAS B only.

Age	<65		>=65	
Years of Education	<12	>=12	<12	>=12
ECAS Total	87	103	88	97
ALS-Specific	66	74	58	74
Language	23	24	20	24
Verbal Fluency	13	15	17	13
Executive	28	31	22	31
ALS Non-Specific	23	27	21	20
Memory	11	16	6	12
Visuospatial	10	10	9	10

Supplemental table 5. Abnormality cut-offs for ECAS Version C, based on healthy Irish controls who completed ECAS C only.

Age	<65		>=65	
Years of Education	<12	>=12	<12	>=12
ECAS Total	98	106	84	80
ALS-Specific	64	78	62	58
Language	24	25	21	19
Verbal Fluency	10	14	12	10
Executive	30	33	25	23
ALS Non-Specific	23	25	21	19
Memory	13	15	10	10
Visuospatial	10	9	10	9

Supplementary table 6. Longitudinal sub-model summary of neuropsychological performance over time.

Outcome	Predictor	β	95% CI	p	Outcome	Predictor	β	95% CI	p
ECAS total	Time (spline 1)	0.45	-0.04 – 0.94	.07	ECAS ALS Specific	Time (spline 1)	0.12	-0.58 – 0.80	.69
	Time (spline 2)	0.30	-0.24 – 0.81	.27		Time (spline 2)	0.05	-1.06 – 1.11	.89
	Age	-0.03	-0.04 – -0.02	<.001		Age	-0.03	-0.03 – -0.01	<.001
	Cognitive reserve	0.37	0.11 – 0.64	.003		Cognitive reserve	0.40	0.21 – 0.59	<.001
	CR x time (spline 1)	-0.03	-0.73 – 0.68	.92		CR x time (spline 1)	-0.15	-1.08 – -0.81	.74
	CR x time (spline 2)	0.04	-0.75 – 0.82	.94		CR x time (spline 2)	-0.17	-1.65 – 1.35	.81
ECAS ALS	Time (spline 1)	0.34	-0.19 – 0.90	.21	RMET	Time (spline 1)	0.25	-0.49 – 0.96	.51
Non-Specific	Time (spline 2)	-1.98	-2.07 – -1.86	<.001	RMET	Time (spline 2)	-0.07	-0.35 – 0.23	.62
	Age	-0.02	-0.03 – -0.01	<.001		Age	-0.05	-0.07 – -0.03	<.001
	Cognitive reserve	0.26	0.06 – 0.45	.01		Cognitive reserve	0.66	0.26 – 1.04	.002
	CR x time (spline 1)	0.73	-0.05 – 1.51	.07		CR x time (spline 1)	-0.25	-1.33 – 0.81	.63
	CR x time (spline 2)	2.15	1.99 – 2.33	<.001		CR x time (spline 2)	-0.15	-0.59 – 0.28	.43
Verbal	Time (spline 1)	-0.20	-1.55 – 1.13	.77	Verbal	Time (spline 1)	0.95	0.04 – 1.80	.05
Fluency (Unrestricted)	Time (spline 2)	0.07	-0.52 – 0.66	.82	Fluency (Restricted)	Time (spline 2)	-0.05	-0.76 – 0.61	.89
	Age	-0.03	-0.05 – -0.01	.005		Age	-0.02	-0.05 – 0.01	.32
	Cognitive reserve	0.49	-0.15 – 1.16	.13		Cognitive reserve	0.61	-0.73 – 1.99	.39
	CR x time (spline 1)	-0.27	-2.22 – 1.69	.78		CR x time (spline 1)	-0.89	-2.19 – 0.34	.17
	CR x time (spline 2)	-0.26	-1.08 – 0.58	.54		CR x time (spline 2)	0.07	-0.94 – 1.10	.89
CWIT	Time (spline 1)	-0.48	-0.87 – -0.07	.03	BNT	Time (spline 1)	-0.25	-0.95 – 0.45	.48
Inhibition	Time (spline 2)	0.44	-0.20 – 1.12	.16	BNT	Time (spline 2)	-0.26	-1.08 – 0.53	.51
	Age	-0.08	-0.11 – -0.05	<.001		Age	-0.03	-0.05 – -0.01	.001
	Cognitive reserve	0.87	0.27 – 1.49	.01		Cognitive reserve	0.55	0.13 – 0.98	.01
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CWIT	Time	-1.04	4.00 – 1.93	.50	RAVLT total	Time	-1.49	-9.86 – 6.63	.72
Switching *	Age	-0.06	-0.09 – -0.04	<.001	*	Age	-0.04	-0.06 – -0.02	<.001
	Cognitive reserve	0.63	0.17 – 1.07	.008		Cognitive reserve	0.29	-0.02 – 0.59	.07
	CR x time	0.56	-3.12 – 4.26	.78		CR x time	-4.49	-14.99 – 6.55	.41

RAVLT delayed	Time (spline 1)	0.11	-0.23 – 0.44	.50	LM Immediate	Time (spline 1)	0.05	-0.34 – 0.49	.84
	Time (spline 2)	-0.08	-1.22 – 1.05	.91		Time (spline 2)	-0.57	-0.96 – -0.14	.009
	Age	-0.04	-0.06 – -0.02	<.001		Age	-0.04	-0.06 – -0.02	.002
	Cognitive reserve	0.21	-0.15 – 0.58	.25		Cognitive reserve	0.38	0.03 – 0.72	.04
	CR x time (spline 1)	-0.27	-0.72 – 0.17	.22		CR x time (spline 1)	-0.73	-1.32 – -0.17	.02
	CR x time (spline 2)	-0.52	-1.84 – 1.01	.57		CR x time (spline 2)	0.20	-0.30 – 0.72	.44
LM Delayed	Time (spline 1)	0.11	-0.23 – 0.44	.50					
	Time (spline 2)	-0.08	-1.22 – 1.05	.91					
	Age	-0.04	-0.06 – -0.02	<.001					
	Cognitive reserve	0.21	-0.15 – 0.58	.25					
	CR x time (spline 1)	-0.27	-0.72 – 0.17	.22					
	CR x time (spline 2)	-0.52	-1.84 – 1.01	.57					

* Splines not added as they did not improve the fit of the model

Supplemental analysis – Joint model specification

Joint Model Specification

Joint longitudinal and time-to-event models are composed of two sub-models, a Cox survival model and a longitudinal mixed effects model. The dependence between the two sub-models is captured through the association structure. In this case a current value association structure was chosen, which assumes the log hazard function of the event at time t is linearly associated with the longitudinal sub-model predictor at time t.

Cox survival model

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The Cox survival model is:

$$h_i(t) = h^0(t) \times \exp (\alpha^1 \times m_i(t) + b^1 x^1_i + b^2 x^2_i + b^3 x^3_i + b^4 x^4_i)$$

or

$$h_i(t) = h^0(t) \times \exp (\alpha^1 * m_i(t) + b^1(\text{age}_i) + b^2(\text{diagnostic delay}_i) + b^3(\text{site of onset}_i) + b^4(\text{C9orf72}_i))$$

where: (t) is survival time, h(t) is the hazard function determined by covariates x^1, x^2, x^3, x^4 . h^0 is the baseline hazard where x^1, x^2, x^3 and x^4 equal 0.

α^1 is the association parameter, representing the strength of association between the longitudinal outcome measure (i.e. cognitive score) and the time-to-event outcome.

x^1 = age, x^2 = diagnostic delay, x^3 = site of onset, x^4 = C9orf72 status. The coefficients b^1, b^2, b^3, b^4 measure the effect size of each covariate.

Longitudinal Mixed Effects Model

Longitudinal mixed effects models were fit for each outcome measure. Time was defined by months since baseline assessment. Natural cubic splines with two degrees of freedom were added to cater for non-linear trends over time. Age, CR and a time by CR interaction were included as fixed effects. Random intercepts and random slopes for time were included. The mixed effects equation for each outcome measure is:

$$y = \beta^0 + \beta^1 x^1_i + \beta^2 x^2_i + \beta^3 x^3_i + \beta^4 x^4_i + \epsilon_i$$

or

$$y = \beta^0 + \beta^1 (\text{time}_i) + \beta^2(\text{age}_i) + \beta^3(\text{CR}_i) + \beta^4(\text{CR}*\text{time}_i) + \varepsilon_i$$

where $x^1 = \text{time}$, $x^2 = \text{age}$, $x^3 = \text{CR}$, $x^4 = \text{CR by time interaction}$, $\varepsilon = \text{random error}$.

Models were evaluated comparing Akaike's information criterion (AIC),

Bayesian information criterion (BIC) and log likelihood ratio test.