Genetic Epidemiology and Cognitive Endophenotyping in Amyotrophic Lateral Sclerosis

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

Marie Ryan
M.B. B.Ch. B.A.O., MRCPI

Supervisor:
Professor Orla Hardiman

2021


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.

I agree to deposit this thesis in the University’s open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

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

Signed: __________________________

Marie Ryan
This thesis is dedicated to Family,
mine and all those affected by ALS
Acknowledgement

First, I would like to thank Professor Orla Hardiman for living up to her reputation as “the rockstar of Irish neurology”. She has been a huge inspiration to me and showed me how it’s possible to change the world for the better. I want to thank her for giving me the wonderful opportunity to conduct important work, for sharing with me her ideas and enthusiasm and for pushing me to exceed what I thought was possible. In addition, I would also like to thank Professor Niall Pender for his ideas and advice, and offering me the opportunity to work with patients and families with other neurodegenerative disorders. Furthermore, I would like to Professor Russell McLaughlin for his countless advice on all matters genetics, and in particular for teaching me everything I know about heritability. I hope one day to achieve his level of skill for explaining complex ideas in simple terms.

I would like to thank Mark Heverin and Bernie Corr for their practical advice and support. Their ability to recall all the people with ALS they have met over the years speaks to how much they care about the work they do and the care they provide. This project would not have been possible without the assistance of Caoifa Madden, Katie Nolan, Bronagh Donohoe and Caoimhe Kavanagh who helped with all the data collection. In particular, I want to thank Emmet Costello for being a wonderful collaborator on this project. I was very fortunate when he decided to transition his research assistant role into a PhD. Whatever he decides to do in the future, I know his hard-work, intelligence and humour will take him far.

I have been honoured to work with the MND Research Team in Trinity College Dublin who opened my eyes to the multitude of ways we can approach complex problem solving. In particular, I want to thank the Irish ALS Register team (Nicola Davis, Michaela Hammond, Colm Peelo), the psychology team (particularly Sile Carney and Marta Pinto Grau; thanks for all the advice and laughs), James Rooney (for answering my many statistics questions), and all the team in the Smurfit Institute of Genetics and the clinical trials team in the RCSI Smurfit Building, particularly Liz Fogarty for running a tight ship. I also want to thank my collaborators in the University of Edinburgh (Professor Sharon Abrahams and Caroline McHutchison) and everyone from the Latin American Epidemiological Network for ALS Consortium for the ideas, advice and support. I thank Science Foundation Ireland and Research Motor Neurone for funding this project.

For achieving the feat of making clinic day the highlight of the week, I want to thank Amina Coffey and Sinead Maguire; Amina for her infectious good humour and Sinead for her wonderful stories. I want to thank them for all their support, particularly in moments
of crisis. Their personal strengths are an inspiration and I look forward to sharing many more coffees together in the future. I would also like to thank the rest of the multidisciplinary team, particularly Sarah Darcy, Eoin Finegan, Aisling O’Reilly, Olivia Grogan and all the IMNDA nurses for showing me what can be achieved with team work, hard work and dedication.

None of what I achieved would ever have been possible without the love and support of my family. The central role you play in my life was a huge influence on my decision to pursue this specific project. In particular, I would like to express thanks to my mum Ann (for endowing me with her sense of curiosity and proving that love is a verb, not a noun), to my dad Michael (for the love of discussing ideas, something which is best done close to midnight), to my granny Kathleen (for teaching me the importance of eating well and sleeping well), to my sisters; Carina (for her advice and support), Aileen (for always believing in me), Louise (for challenging me to do my best and the patience she’s going to need) and Fiona (to whom I extend the highest compliment that she is just like her granny); and to my niece and nephews (for all the joy they bring me). I would also like to thank my extended family and my friends Clare O’Neill, Caitriona Fahy, Michelle Delaney and Denise Howard for all the love, support and good times over the years.

Voltaire is believed is to described doctors ‘as people who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing’. I would like to finish this section by expressing my heartfelt thanks to all the relatives who participated in this project. Their time and dedication and the stories they have shared have given me some insight into the many worlds of people with ALS. I hope that this work has furthered our understanding the nature of the disease and will be used as a springboard to identify potential treatments for this disease devastating their families.
Abstract

Amyotrophic Lateral Sclerosis (ALS) is a progressive debilitating and ultimately fatal neurodegenerative disorder affecting both upper (UMN) and lower (LMN) motor neurons. While it has been long known to exist on a spectrum of motor and cognitive dysfunction, recent genetic and epidemiological studies have also found a significant overlap between ALS and other neuropsychiatric disorders. While the exploration of this spectrum of liability is still in its infancy, delineating the nature of these phenotype-genotype correlations may provide us with a novel means for categorising these disorders as profiles of multi-endophenotypic traits and their associated genetic determinants. This is hoped will provide insight into the pathogenesis of both ALS and associated disorders, ultimately assisting in the identification of novel targets for drug development.

In this project, a “clan genomics” model was applied to deconstruct this shared genetic liability into population level and family specific genetic risk. The influence of population-specific genetic signatures on ALS risk and phenotype was explored both within the genetically homogenous Irish population and across Irish and Latin-American clinic-based cohorts, adding to our knowledge of ALS epidemiology in these understudied regions. In the largest such study conducted to date, ALS heritability estimates showed that genetic and environmental factors contribute approximately equally to variation in ALS risk. For the first time, it was shown that genetic factors play a greater role in ALS risk among women. A significantly younger age of onset was observed among Cuban ALS patients, not accounted for by known ALS genes or demographic factors. This suggests that larger scale genome-phenotype correlation consortium efforts may be highly informative in identifying rare large-effect variants in Cuban ALS kindreds and in exploring epigenetic interactions in these regions.

How ALS risk manifests within kindreds was explored in three studies. Analysis of Irish ALS register data showed that, in Ireland, at least 20% of ALS is familial and could be much higher if extended phenotypes associated with ALS were also considered. However, while the importance of extended phenotypes within ALS kindreds is increasingly recognised, there remains a lack of clarity as to how best to define significant familial clustering of these disorders. The largest ALS family aggregation study performed to date confirmed that relatives of those with ALS are at risk of developing many neuropsychiatric disorders, particularly FTD and schizophrenia. These data were used in conjunction with novel probability modelling approaches to propose new criteria for redefining familial ALS to encompass significant clustering of FTD or schizophrenia. Similar to more traditionally defined familial ALS, probands from these ALS and neuropsychiatric familial clusters also developed ALS at a younger age, evidencing the
clinical relevance of the high shared genetic risk burden within these families. That this increased genetic risk was not entirely attributable to the pathogenic C9orf72 repeat expansion, suggests that other rare, pleiotropic gene variants are yet to be discovered in the Irish population.

This is further supported by the findings of extensive cognitive and neuropsychiatric changes among relatives of people with ALS. Both asymptomatic carriers of the pathogenic C9orf72 repeat expansion and their non-carrier kindred, showed changes mirroring patterns seen in ALS patients. In particular, clustering of both verbal fluency deficits and initiation apathy in C9orf72 negative relatives from C9orf72 kindreds infers the existence of selective neural networks vulnerabilities in this cohort. While this may reflect additional unidentified genetic risk in this cohorts, aligning with the oligogenic model of ALS, how epigenetic factors contribute to this clustering will need to be explored in future studies. Nonetheless, this current work raises concerns as to the common practice of using asymptomatic non-carriers as the only controls in studies of pre-symptomatic mutation carriers.

Finally, much of the work of this project has direct applications for ALS genetic counselling. Furthermore, this work has contributed significantly to our understanding of ALS genetic epidemiology and provides strong support in favour of the hypothesis that ALS is a disorder of aberrant neural networks. It has become increasingly clear that a simple neurodevelopmental or neurodegenerative model does not best fit ALS. Instead, both genetic and environmental risk can be considered under a ‘Development Risk Factor Model of ALS’, where genetic or time sensitive environmental exposures result in the development of vulnerable neural networks, whose phenotypic manifestations may interact with environmental factors over a lifetime, ultimately resulting in decompensation and the development of overt symptomology. As such, high risk family units may play an essential role in future research teasing out the risk architecture of ALS, by allowing for the exploration of how epigenetic interactions with kindreds may alter phenotypic disease manifestations. Developing biomarker profiles indicating dysregulation in specific neural networks will facilitate the implementation of timely and personalised interventions which will hopefully lead to substantial improvements in outcomes.
Peer-reviewed publications from this thesis


Presentations regarding this thesis

Virtual Conference

1. “Neuropsychological dysfunction in asymptomatic ALS relatives: A marker of disease risk?”. Ryan M et al. Accepted at European Network to Cure ALS (ENCALS), May 2021

2. “Genetic modifiers and phenotypic heterogeneity within ALS kindreds”. Ryan M et al. Accepted at 31st International Symposium on ALS/MND, December 2020

3. “Cognitive dysfunction as an endophenotype in pre-symptomatic C9orf72 repeat expansion carriers”. Ryan M et al. Accepted at American Academy of Neurology (AAN) Annual Meeting, April 2020

Oral presentations:

1. “Cognitive dysfunction as an endophenotype in pre-symptomatic C9orf72 repeat expansion carriers”. Ryan M et al. Accepted at 30th International Symposium on ALS/MND, Perth, December 2019

2. “Cognitive dysfunction in pre-symptomatic C9orf72 repeat expansion carriers”. Ryan M et al. Accepted at IICN Registrar’s Prize in Clinical Neuroscience Meeting, Dublin, November 2019

3. “Executive impairment among C9orf72 repeat expansion carriers: An under recognized phenomenon”. Ryan M et al. Accepted at European Network to Cure ALS (ENCALS), Tours, May 2019


5. “Heritability of ALS in Irish population”. Ryan M et al. Accepted at IICN Registrar's Prize in Clinical Neuroscience Meeting, Dublin, November 2018

6. “Oligogenic and discordant inheritance: A population based genomic study of Irish kindreds carrying the C9orf72 repeat expansion”. Ryan M et al. Accepted at European Network to Cure ALS (ENCALS), Oxford, June 2018
7. “Oligogenic and discordant inheritance: A population based genomic study of Irish kindreds carrying the C9orf72 repeat expansion”. Ryan M et al. Accepted at 54th Annual Irish Neurological Association Meeting, Belfast, May 2018

E-presentations:

1. “Oligogenic and discordant inheritance: A population based genomic study of Irish kindreds carrying the C9orf72 repeat expansion” Ryan M et al. Accepted at 4th European Association of Neurology Congress, Lisbon, June 2018
2. “Frequency of Neuropsychiatric Disorders in First and Second-degree relatives of patients with Amyotrophic Lateral Sclerosis (ALS)”. Ryan M et al. Accepted at 3rd European Association of Neurology Congress, Amsterdam, June 2017

Poster presentations:

1. Beyond Known Genes: The Relevance of Detailed Family Histories. Ryan M et al. Accepted at 29th International ALS Symposium, Glasgow, December 2018

2. Determining the risk of ALS in relatives of patients with ALS: A study of re-categorisation rates from “sporadic ALS” to “familial ALS”. Ryan M et al. Accepted at European Network to Cure ALS (ENCALS), Oxford, June 2018

3. Determining the risk of ALS in relatives of patients with ALS: A study of re-categorisation rates from “sporadic ALS” to “familial ALS”. Ryan M et al. Accepted at 54th Annual Irish Neurological Association Meeting, Belfast, May 2018

4. Frequency of Neuropsychiatric symptoms in First and Second-degree relatives of patients with Amyotrophic Lateral Sclerosis (ALS). Ryan M. et al. Accepted at 28th International ALS Symposium, Boston, December 2017

5. Frequency of Cognitive Impairment in First and Second-degree relatives of patients with Amyotrophic Lateral Sclerosis (ALS). Ryan M. et al. Accepted at 28th International ALS Symposium, Boston, December 2017
# Table of Contents

1. **Chapter 1: An introduction to Motor Neurone Disease** ......................................................... 27
   1.1. Introduction .......................................................................................................................... 27
   1.2. Motor Neurone Diseases ...................................................................................................... 30
   1.3. Para-clinical investigations ................................................................................................. 34
   1.4. Clinical Management of ALS .............................................................................................. 36
   1.5. ALS and FTD: A clinical spectrum? ....................................................................................... 45
   1.6. Genetics of ALS: Introduction ............................................................................................ 46
   1.7. Neuropathology ..................................................................................................................... 50
   1.8. Neural networks in ALS ....................................................................................................... 54
   1.9. Cognitive and behavioural changes in ALS ......................................................................... 56
   1.10. Disease progression and staging in ALS ............................................................................. 63
   1.11. ALS Epidemiology ............................................................................................................. 65
   1.12. The ‘Familial’/‘Sporadic’ ALS dichotomy .......................................................................... 67
   1.13. Genetic architecture of ALS and “Clan Genomics” .......................................................... 68
   1.14. Genotype-phenotype confounders ..................................................................................... 70
   1.15. Environmental risk factors for ALS ................................................................................... 74
   1.16. Multistep model of ALS ...................................................................................................... 77
   1.17. Conclusion .......................................................................................................................... 78
2. **Chapter 2: Clan Genomics in ALS: The Architecture of Genetic Risk** ..................................... 79
   2.1. Introduction .......................................................................................................................... 79
   2.2. Population Genetics and ALS Risk ..................................................................................... 80
   2.3. Genetic Burden within ALS kindreds ................................................................................... 84
   2.4. Novel Endophenotypic Approaches in ALS ......................................................................... 88
   2.5. Summary of findings ............................................................................................................ 95
   2.6. Limitations of research studies on asymptomatic mutation carriers ................................. 96
   2.7. Lessons in the search for novel neuropsychological endophenotypes in ALS ............... 98
   2.8. Future Directions ................................................................................................................ 99
12. Chapter 12: Results Part VIII: Comparison of Neuropsychiatric Traits of Relatives of People with ALS and Healthy Controls

12.1. Introduction

12.2. Demographics

12.3. Comparison of neuropsychiatric traits of relatives and controls

12.4. Comparison of neuropsychiatric traits of familial ALS relatives, sporadic ALS relatives and controls

12.5. Controlling for impact of age on neuropsychiatric traits

12.6. C9orf72 positive relatives

12.7. Corf72 negative relatives from C9orf72 kindreds

12.8. Summary of findings

12.9. Discussion

13. Chapter 13: Summary of Findings and Discussion, Conclusions, Limitations and Future Directions

13.1. Introduction

13.2. Advancing ALS epidemiology

13.3. Sex-specific disease liability

13.4. ‘Development Risk Factor Model of ALS’

13.5. Conclusion

13.6. Project Limitations

13.7. Future directions

14. References
List of Figures

Figure 1-1: The genetic and pathological overlap of ALS and FTD .......................................................... 51
Figure 1-2: Cellular mechanisms of toxicity in ALS/FTD ............................................................................. 53
Figure 1-3: Three non-exclusive mechanisms have been proposed for C9orf72 associated ALS/FTD ................................................................................................................................. 54
Figure 1-4: Clan Genomics .......................................................................................................................... 70
Figure 2-1: Endophenotypes as measures of Intermediate Disease Liability ............................................ 90
Figure 2-2: Criteria for defining endophenotypes ....................................................................................... 90
Figure 4-1: Byrne criteria for familial amyotrophic lateral sclerosis (FALS) ........................................... 111
Figure 5-1: Liability Model for two distinct populations ........................................................................ 144
Figure 5-2: Mathematical terminology in relation to the liability model for two distinct populations ................................................................................................................................. 148
Figure 5-3: Sex-specific heritability estimates by overall heritability cohort and C9orf72 negative sub-cohort ........................................................................................................................................ 155
Figure 6-1. A). Kaplan-Meier plots of survival probabilities stratified by nationality for 1) onset to death and 2) diagnosis to death. B) Kaplan-Meier plot of survival probabilities for Cuban ALS population stratified by ethnicity for 1) onset to death and 2) diagnosis to death .......................................................... 164
Figure 7-1: Flow chart of patients who met inclusion/exclusion criteria for the study .............................. 175
Figure 7-2: Temporal trends on age-standardised Familial ALS incidence ................................................. 178
Figure 9-1: Mean ages of onset and diagnosis by familial cohort ........................................................ 208
Figure 9-2: Proportion of familial ALS probands who carry the pathogenic C9orf72 repeat expansion ............................................................................................................................................ 209
Figure 10-1: Flow chart of recruitment for ALS relatives studies ............................................................ 217
Figure 10-2: Comparison of ECAS verbal fluency across groups, by C9orf72 and kindred status .............................................................. ........................................................................ 222
Figure 10-3: Comparison of ECAS performance scores across familial ALS relatives, sporadic ALS relatives and control cohorts, by age category ........................................................................... 226
Figure 10-4: ECAS performance of C9orf72 positive relatives by age category (<45 years, 45-54 years, 55-64 years) ................................................................................................................ 229
Figure 10-5: ECAS performance of C9orf72 negative relatives from C9orf72 kindreds, by age category (<45 years, 45-54 years, 55-64 years and 65+ years) ............................................................. 231
Figure 11-1: Comparison of ALS relatives and control performance on Iowa Gambling Task and Rey Auditory Verbal Learning Test .................................................................................................... 240
Figure 11-2: Comparison of verbal fluency performance across C9orf72 positive relatives, C9orf72 negative relatives and controls ................................................................. 244
Figure 11-3: Comparison of memory task performance across C9orf72 positive relatives, C9orf72 negative relatives and controls ................................................................. 245
Figure 11-4: Comparison of Boston Naming Test performance across C9orf72 positive relatives, C9orf72 negative relatives and controls ................................................................. 246
Figure 11-5: Comparison of Iowa Gambling Task performance across C9orf72 positive relatives, C9orf72 negative relatives and control ................................................................. 247
Figure 11-6: Comparison of Intellectual Functioning performance across C9orf72 positive relatives, C9orf72 negative relatives and controls................................................................. 248
Figure 12-1: C9orf72 positive relative performance on Barrett Impulsiveness (BIS) Self-Control and Cognitive Complexity and Ten Item Personality Inventory Extraversion ...................... 260
Figure 12-2: Performance of C9orf72 negative relatives from C9orf72 kindreds, by age category, on Generalised Anxiety Disorder-7 (GAD-7) and Ten Item Personality Inventory (TIPI) Openness to Experience ........................................................................................................ 262
Figure 13-1: Threshold of liability and cognitive endophenotypes in ALS .......................... 275
Figure 13-2: Family Centered Care Model. ..................................................................... 281
List of Tables

Table 1-1 Revised El Escorial Criteria. ................................................................. 29
Table 1-2: Clinical and demographic characteristics of patients on Irish ALS register diagnosed between Jan 1, 1995 – Dec 31, 2018...................................................................................... 31
Table 1-3: Key differential diagnosis subcategorised by core clinical features15........... 33
Table 1-4: Investigations to consider in the work-up of ALS ........................................ 34
Table 1-5 ALS associated genes. .............................................................................. 46
Table 1-6: International Consensus Criteria for behavioural variant FTD (bvFTD). ............ 59
Table 1-7: Diagnostic classification of frontotemporal syndromes in ALS....................... 60
Table 1-8: Evidence for putative environmental risk factors in ALS............................. 75
Table 4-1: Neuropsychological and neuropsychiatric assessment tools ....................... 117
Table 4-2: The structure of the UK Biobank ‘Thoughts and Feelings’ Questionnaire............ 132
Table 5-1: Description of key concepts discussed with explanation and justification of methodological approaches used in this study ______________________________________ 149
Table 5-2. Comparison of demographic and clinical characteristics between overall study cohort (1995-2017) and heritability sub-cohort (2008-2017).............................................................. 152
Table 5-3. Parent-offspring ALS concordance rates by overall heritability cohort and C9orf72 negative sub-cohort .................................................................................... 153
Table 6-1. Comparison of demographic and clinical characteristics by population origin ..... 164
Table 6-2: Comparison of variables by self-reported ethnicity (Cuba)............................. 166
Table 6-3: Known ALS-associated variants in Cuban Population .................................... 167
Table 6-4: Age of onset and survival periods for Hispanic/Latino clinic-based populations ... 168
Table 8-1: Demographic and clinical details of patients included or not included in Family Aggregation Study 2015-2017................................................................. 183
Table 8-2: Comparison of frequency of neurological or neuropsychiatric disorders among relatives of ALS patients versus controls (Family Aggregation Study 2015-2017)........ 185
Table 8-3: Comparison of demographic and clinical details of patients included in each family aggregation study................................................................. 186
Table 8-4: Comparison of reported results from each family aggregation study ............. 187
Table 8-5: Comparison of frequency of neurological or neuropsychiatric disorders among relatives of ALS patients versus controls (Pooled Family Aggregation Study 2008-2017) ....... 189
Table 8-6: Relative risk of schizophrenia and FTD in relatives of C9orf72 positive and negative ALS probands ...................................................................................... 190
Table 9-1: Kindred size at or above which the probability of member(s) developing a disorder exceeds 5% ........................................................................................................................................ 199
Table 9-2: Probability of having exactly this number of relatives with ALS ........................................... 200
Table 9-3: Probability of having exactly this number of relatives with FTD ........................................... 200
Table 9-4: Probability of having exactly this number of relatives with schizophrenia ..................... 201
Table 9-5: Probability of having exactly this number of relatives with dementia (unspecified) ........... 201
Table 9-6: Total and average number of affected relatives with specified disorders, from pooled family aggregation study (2008-2017) ......................................................................................................................... 202
Table 9-7: Poisson distribution for probability of first- and/or second-degree relative developing ALS, FTD or schizophrenia ........................................................................................................ 205
Table 9-8: Comparison of demographic and clinical characteristics by familial status ....................... 208
Table 9-9: Comparison of mean ages of onset and diagnosis by familial status (C9orf72 carriers excluded) ............................................................................................................................................. 210
Table 10-1: Comparison of ECAS performance across familial ALS relatives, sporadic ALS relatives and controls ........................................................................................................................................ 219
Table 10-2: Comparison of performance on ECAS sub-tests (language and verbal fluency) across familial ALS relatives, sporadic ALS relatives and controls ........................................ 219
Table 10-3: Comparison of ECAS performance across C9orf72 positive relatives, C9orf72 negative relatives and controls ........................................................................................................ 221
Table 10-4: Comparison of ECAS performance scores across familial ALS relatives, sporadic ALS relatives and control cohorts by age category .................................................................................. 225
Table 10-5: Jonckheere-Terpstra tests of ECAS performance across increasing age categories (<45 years, 45-54 years, 55-64 years and 65 years or more) ................................................................................ 227
Table 11-1: Comparison of participants and non-participants for full neuropsychological assessment ........................................................................................................................................... 237
Table 11-2: Comparison of standardised neuropsychological performance of relatives of people with ALS and controls ........................................................................................................... 238
Table 11-3: Significant differences in neuropsychological performance across familial ALS relatives, sporadic ALS relatives and control groups .............................................................................. 241
Table 12-1: Comparison of participants and non-participants for online neuropsychiatric trait assessment ..................................................................................................................................... 254
Table 12-2: Comparison of Neuropsychiatric Traits Assessment performance among relatives of ALS patients and controls .............................................................................................................. 255
Table 12-3: Significant differences in neuropsychiatric traits across familial ALS relatives, sporadic ALS relatives and controls ................................................................................................. 257
Table 12-4: Number of participants in each age category, by C9orf72 and kindred status ..... 258
Glossary of terms

ALS – Amyotrophic Lateral Sclerosis
AQ – Autism Spectrum Quotient
ASRS – Adult ADHD Self-Report Scale
AUDIT – Alcohol Use Disorders Identification Test
BBI – Beaumont Behavioural Index
BIS – Barrett Impulsiveness Scale
CAPE – Community Assessment of Psychic Experiences
DAS – Dimensional Apathy Scale
ECAS – Edinburgh Cognitive and Behavioural Screen
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
IGT – Iowa Gambling Task
OCI-R – Obsessive-Compulsive Inventory Revised
PALPA – Psycholinguistic Assessment of Language Processing Abilities
PHQ – Patients Health Questionnaire
PLE – Psychotic-like experience
PSQ – Psychosis Screening Questionnaire
RAVLT – Rey Auditory Verbal Learning Test
RCFT – Rey Complex Figure Test
SALS – Sporadic ALS
SART – Sustained Attention to Response Task
TIPI – Ten Item Personality Inventory
TOPF – Test of Premorbid Functioning
WAIS – Wechsler Adult Intelligence Scale
WMS – Wechsler Memory Scale
1. Chapter 1: An introduction to Motor Neurone Disease

1.1. Introduction

Motor neurone disease (MND) is a progressive debilitating and ultimately fatal neurodegenerative disorder affecting both upper (UMN) and lower (LMN) motor neurons. This umbrella term is used to describe several different clinical syndromes, thought to represent a clinical spectrum from predominant upper motor neuron involvement (Primary Lateral Sclerosis [PLS]) to predominant lower motor neuron involvement (Progressive Muscular Atrophy [PMA]). These heterogenous clinical entities are grouped together as they share many overlapping clinical features and pathological hallmarks. PLS, PMA and other associated conditions are discussed in detail in section 1.2. Amyotrophic Lateral Sclerosis (ALS) is the most common motor neurone disease, accounting for over 75% of cases\(^1\). In many parts of the world, the term is used synonymously to describe motor neurone disease. In the US, the name Lou-Gehrig’s disease is often used to refer to ALS in honour of a famous baseball player who developed and ultimately died from this condition. In this thesis, the specific term ALS is used rather than the broader term MND. Where the term MND is used, it refers to full clinical spectrum of associated diseases.

The focus is on ALS as it is both the most common form of MND and has the worst prognosis, with most people with the condition living just two to three years after diagnosis\(^2\). ALS is an age-related neurodegenerative disorder. As such, with the world’s population aging, the crude incidence of ALS is expected to increase, translating to increased social, economic and healthcare related challenges associated with caring for individuals with the disease. Finally, despite the bleak prognosis, there is a startling lack of effective treatments for the condition. ALS is an heterogenous condition in many regards, with marked differences observable between individual patients with regard to phenotypic manifestation and disease progression and survival. Many researchers in the field believe now propose that the condition should be stratified into multiple sub-groups based on phenotypic and genetic features. The hope is that such sub-phenotypes would improve the homogeneity of trial cohorts, making it easier to identify effective treatments for sub-cohorts of ALS patients.
1.1.1. ALS Clinical Features

ALS presents with numerous symptoms and signs associated with both upper and lower motor neuron degeneration. UMN involvement is suggested by the presence of hypertonicity and brisk reflexes, while muscle wasting, fasciculations and reduced reflexes imply LMN degeneration. The condition is often sub-categorized by its motor site of onset (limb, bulbar or respiratory), although extra-motor involvement is increasingly recognised. Approximately 70% of cases are spinal onset, presenting with painless, progressive limb weakness, which is often focal, distal and asymmetrical in its earliest stages. In the upper limbs, the disease can cause loss of functional hand dexterity and poor grip, associated with wasting of intrinsic small muscles of the hand. Proximal weakness may occur, with patients more likely to report the presence of fasciculations in the larger proximal muscles. In the lower limbs, patients may report difficulty with walking, foot drop, difficulty rising from chairs/climbing stairs or heaviness and/or stiffness in one or both legs. Muscle wasting (particularly of the tibialis anterior), fasciculations and/or spasticity may be noted on clinical examination.

Bulbar onset occurs in approximately 20 – 30% of cases and is associated with symptoms of dysarthria or dysphonia, dysphagia (more prominent for liquids than solids) and sialorrhea. Associated clinical findings include tongue weakness with spasticity or wasting and fasciculations. UMN involvement is suggested by a pathologically brisk jaw jerk. Respiratory onset occurs rarely (about 2% of cases), presenting as early dyspnoea, orthopnoea or hypercapnic features from hypoventilation overnight such as hypersomnolence, morning headaches and reduced exercise tolerance. Patients may also report symptoms consistent with weakness of the posterior neck and thoracic paraspinals. Finally, while extra-motor involvement including mild sensory or cognitive disturbance, dysautonomia and emotional lability is seen in ALS, prominent extra-motor symptoms should prompt consideration of alternative diagnoses.

1.1.2. Diagnostic criteria for ALS

The El Escorial consensus criteria for the diagnosis of ALS were first proposed in 1994 and revised in 1999 to increase their sensitivity. In the revised criteria, patients are categorised along a spectrum of probability from “possible” to “definite” ALS based on the number and specific bodily regions affected, the involvement of upper and/or lower motor neurones and the presence or absence of supportive neurophysiological findings (Table 1-1). These criteria were proposed to provide a formal framework for the diagnosis of ALS. This allows for increased diagnostic certainty by refining the core clinical features.
from the complex clinical presentation, thus allowing for the standardisation of patients to be included in multi-centre clinical trials\(^5\). Recognition of patients with “possible” ALS allows for the inclusion of those with only region affected (if both UMN and LMN signs are present). This permits those in the earliest clinically detectable stages of disease to partake in clinical trials without increasing the risk of including those with erroneous diagnoses\(^5\). Yet, critics of the framework would argue that it is unclear whether the objectives of improving diagnostic accuracy and reducing diagnostic delay have been achieved\(^5\). Furthermore, they highlight the limitations of the framework in regard that the exclusion of cognitive MND sub-phenotypes, in turn limits the inclusion of these sub-phenotypes in clinical trials.

Table 1-1 Revised El Escorial Criteria.

*From Brooks et al (2000)*\(^4\)

<table>
<thead>
<tr>
<th><strong>Definite ALS</strong></th>
<th>Presence of UMN, as well as LMN signs, in the bulbar region and at least two spinal regions or Presence of UMN and LMN signs in three spinal regions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Probable ALS</strong></td>
<td>Presence of UMN and LMN signs in at least two regions with some UMN signs necessarily rostral to (above) the LMN signs.</td>
</tr>
<tr>
<td><strong>Probable ALS – Laboratory supported</strong></td>
<td>Presence of UMN and LMN signs in only one region, or Presence of UMN signs alone in one region, and LMN signs defined by EMG criteria are present in at least two regions(^a)</td>
</tr>
<tr>
<td><strong>Possible ALS</strong></td>
<td>Presence of UMN and LMN signs in only one region or Presence of UMN signs in two or more regions or Presence of LMN signs rostral to UMN signs and the diagnosis of Probable ALS – Laboratory supported cannot be proven(^b)</td>
</tr>
</tbody>
</table>

\(^a\): Also requires proper application of neuroimaging and clinical laboratory protocols to exclude other causes; \(^b\): Other diagnoses must have been excluded to accept a diagnosis of Possible ALS

### 1.1.3. Differential Diagnoses

ALS is a clinical diagnosis, distinguished by its progressive nature and upper and lower motor neuron involvement affecting more than one body region. There are limited potential differential diagnoses for such a presentation. While the more classical and aggressive forms of MND are relatively easy to diagnose, difficulty arises in distinguishing more slowly progressive indolent conditions from MND spectrum disorders such as PLS and PMA. Indeed, often it is how the disease progresses over time, along
with the development of certain key clinical features that assists with making these
distinctions. The key distinguishing clinical features for both motor neurone disease
spectrum disorders and other differential diagnoses for ALS are outlined below and in
Table 1-2.

1.2. Motor Neurone Diseases

1.2.1. Primary Lateral Sclerosis

Primary Lateral Sclerosis (PLS) is an adult-onset disorder characterised by isolated
degeneration of the upper motor neurons. In contrast, to hereditary spastic paraparesis
which may have a similar clinical presentation, patients with PLS are usually older and
are less likely to report a positive family\(^6\). PLS accounts for approximately 1-3\% of all
new diagnoses of MND\(^6\). A cut-off time of four years from symptom onset is suggested
after which patients are less likely to develop lower motor neuron signs, although LMN
signs may appear even after several years\(^6\). Patients with muscle weakness, weight loss,
respiratory dysfunction and bulbar onset disease are more likely to develop LMN signs\(^7\).
PLS carries a better prognosis with increased survival times and high levels of
independence decades after diagnosis. Individuals presenting with an upper motor
neuron predominant syndrome who later develop lower motor neuron involvement are
expected to experience levels of disability similar to those reported in ALS, but slower
progression\(^6\).

1.2.2. Progressive Muscular Atrophy

Progressive muscular atrophy (PMA) is an adult-onset, non-hereditary progressive
disorder with selective involvement of the lower motor neurons. While upper motor
neuron involvement should not initially be present, many patients with PMA may develop
UMN signs at later stages of the disease\(^5,8\). Corticospinal tract involvement was found
in up to 50\% of patients with PMA on autopsy series\(^9\). The prognosis of PMA is similar
to that of ALS with one prospective study of 37 patients with PMA reporting a median
survival from symptom onset of 56 months from time of initial weakness and a 5-year
survival-rate of 45\%\(^8\). Factors predictive of reduced survival were vital capacity at
baseline and decline in vital capacity in first 6 months following diagnosis. Those with
symmetrical distal weakness and segmental weakness have a more favourable
prognosis\(^5,8\).
1.2.3. *Flail Limb Variant ALS*

Segmental, non-progressive variants of ALS are rare and may be isolated to either the upper limbs (Flail arm syndrome/ brachial amyotrophic diplegia) or lower limbs (Flail leg syndrome/ Pseudopolyneuritic ALS). They are characterised by progressive, symmetrical muscle weakness and wasting in the upper and lower limbs respectively, without involvement of any other anatomical region. The pattern of weakness is predominantly proximal in the upper limbs and distal in the lower limbs. Male predominance in these variants is approximately 3-4 times that observed in the ALS population \(^{10}\) and they are associated with slower disease progression and improved survival compared with classical forms of ALS.

1.2.4. *Kennedy’s disease*

Kennedy’s disease, also known as spinal and bulbar muscular atrophy (SBMA), is an X-linked recessive, adult-onset disorder characterised by progressive degeneration of the lower motor neurons. It is caused by a CAG repeat expansion in exon 1 of the androgen receptor gene, with an expansion length of greater than 38 considered pathogenic \(^{11}\). Patients with this disease typically present with proximal lower limb weakness and atrophy, although upper limb and bulbar involvement is reported \(^{12}\). Endocrine disturbances including the development of gynecomastia are the most common systemic manifestations. Metabolic, urinary and cardiac involvement are also reported \(^{11}\). Those with the condition are expected to progress slowly without a significant decline in life expectancy \(^{13}\).

Table 1-2: Clinical and demographic characteristics of patients on Irish ALS register diagnosed between Jan 1, 1995 – Dec 31, 2018

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sex, male (%)</th>
<th>Age of onset, years (^a)</th>
<th>Survival from onset, months (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>2221</td>
<td>1148/2034 (56.4)</td>
<td>63.8 (63.3 – 64.3)</td>
<td>28 (26.9 – 29.1)</td>
</tr>
<tr>
<td>PLS</td>
<td>71</td>
<td>30/69 (43.5)</td>
<td>57.3 (54.7 – 59.9)</td>
<td>212 (NA) (^c)</td>
</tr>
<tr>
<td>UMN ALS</td>
<td>43</td>
<td>17/42 (40.5)</td>
<td>63.5 (60.1 – 66.8)</td>
<td>19 (12.6 – 25.4)</td>
</tr>
<tr>
<td>PMA</td>
<td>50</td>
<td>48/50 (96)</td>
<td>60.0 (56.3 – 63.7)</td>
<td>86 (69.9 – 102.1)</td>
</tr>
<tr>
<td>Kennedy’s disease</td>
<td>11</td>
<td>10/10 (100)</td>
<td>42.6 (36.1 – 49.1)</td>
<td>NA (^d)</td>
</tr>
</tbody>
</table>
ALS: amyotrophic lateral sclerosis. PLS: primary lateral sclerosis. UMN: upper motor neuron. PMA: progressive muscular atrophy. NA: not available. a: mean+95% CI age of onset. b: median+95% CI survival from onset. c: unable to calculate standard error for median survival time as insufficient events occurred. 65.2% of PLS were censored. d: unable to calculate median survival and standard error as insufficient events occurred. 100% of patients with Kennedy’s disease were censored.

1.2.5. **Other differential diagnoses**

It is important to exclude other differential diagnoses for ALS, as in many cases these mimic conditions are treatable. They can be considered in respect to whether the person presented initially with either predominant upper or lower motor neuron involvement (Table 1-3). Reports of significant sensory symptoms, bladder dysfunction, non-neurological symptoms or a strong family history should caution clinicians against the diagnosis of ALS, PLS or PMA. Similarly, involvement of regions not anatomically explained by the proposed pathology (e.g., bulbar signs in those with suspected cervical myeloradiculopathy; UMN signs in those with suspected myopathy/neuropathy) should prompt an expansive reconsideration of the differential diagnosis. Cervical myeloradiculopathy and multifocal motor neuropathy were the two most common ALS mimic syndromes identified in a study of two population-based ALS registers.

While, ALS is classically described as a disorder causing painless weakness, reports of pain secondary to musculoskeletal strain or spasticity and cramps are not uncommon. In contrast, some clinical signs carry a high positive predictive value for ALS including bilateral tongue wasting, head drop and ‘split hand’. Cognitive and behavioural change are seen in over 50% of patients with ALS. While the presence of characteristic impairment in these domains could potentially support the diagnosis of ALS over others, their absence does not exclude the diagnosis.

Finally, an atypical disease course may suggest different underlying pathology. For example, PLS is expected to progress over a longer time period (mean survival 10-20 years) compared with corticobasal degeneration and multiple systems atrophy (mean survival 7 years). While a longer time taken to determine the diagnosis of ALS, is associated with a better prognosis for the individual, delays in confirming the diagnosis can delay access to disease modifying therapies, multidisciplinary care and clinical trial enrolment opportunities.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Classical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mixed UMN and LMN signs</strong></td>
<td></td>
</tr>
<tr>
<td><strong>UMN signs only (Differential diagnosis PLS)</strong></td>
<td></td>
</tr>
<tr>
<td>Hereditary spastic paraparesis (HSP)</td>
<td>Very slowly progressive lower limb spasticity and weakness. Bladder dysfunction. Additional features in some subtypes include peripheral neuropathy, ataxia, dementia, epilepsy, ichthyosis.</td>
</tr>
<tr>
<td>Primary progressive multiple sclerosis (PPMS)</td>
<td>Slowly progressive course. Mostly commonly presents with lower limb spasticity and weakness. Sensory, brainstem, cerebellar, sphincter and visual disturbance expected as disease progresses.</td>
</tr>
<tr>
<td>Metabolic myelopathies (B12 and copper deficiencies, X-linked adrenoleukodystrophy)</td>
<td>Slowly progressive lower limb spasticity and weakness. Sensory impairment. Bladder dysfunction. Family history (X-linked Adrenoleukodystrophy).</td>
</tr>
<tr>
<td><strong>LMN only signs (Differential diagnosis PMA)</strong></td>
<td></td>
</tr>
<tr>
<td>Multifocal motor neuropathy with conduction block</td>
<td>Slowly progressive, asymmetrical and distal weakness with very little wasting.</td>
</tr>
<tr>
<td>Motor-predominant chronic inflammatory demyelinating polyradiculoneuropathy</td>
<td>Relapsing and remitting course, predominantly symmetrical distal muscle weakness.</td>
</tr>
<tr>
<td>Cervical polyradiculopathy (mimics brachial diplegia)</td>
<td>Neck pain radiating unilaterally or bilaterally into the arms with associated paraesthesia, weakness, and sensory loss.</td>
</tr>
<tr>
<td>Radiation-induced radiculopathy</td>
<td>History of radiotherapy to the pelvis and para-aortic lymph nodes for testicular and gynaecological tumours. Lower limb weakness, paraesthesia and sensory loss.</td>
</tr>
<tr>
<td>Benign fasciculations</td>
<td>Fasciculations. Absence of weakness.</td>
</tr>
</tbody>
</table>
Neuralgic amyotrophy | Painful, progressive, usually upper limb weakness and wasting, affecting multiple nerve roots. Symptoms arrest and slowly recover. Absence of trauma, viral illness or vaccination.

Inclusion body myositis | Slowly progressive painless muscle weakness and wasting with a characteristic predilection for wasting of the medial forearm and quadriceps muscles.

Asymmetrical spinal muscular atrophy | Slowly progressive muscle wasting and weakness characteristically involving muscles innervated by the C7–T1 segments. Typically affects young men.

UMN: upper motor neuron; LMN: lower motor neuron; PLS: primary lateral sclerosis; PMA: progressive muscular atrophy.

1.3. Para-clinical investigations

In making the diagnosis of a motor neuron disease spectrum disorder, no investigations are mandatory. Clinicians instead should be guided by their clinical findings, both historical and examination, in determining which differential diagnoses must be excluded. Suggested investigations are listed in Table 1-4 below.

Table 1-4: Investigations to consider in the work-up of ALS

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Differential diagnoses</th>
<th>Expected findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum B12</td>
<td>B12 deficiency</td>
<td>Low serum B12</td>
</tr>
<tr>
<td>Serum Copper</td>
<td>Copper deficiency</td>
<td>Low serum copper</td>
</tr>
<tr>
<td>Serum very long-chain fatty acid</td>
<td>X-linked adrenoleukodystrophy</td>
<td>High serum very long-chain fatty acid</td>
</tr>
<tr>
<td>Serum creatine kinase</td>
<td>Inclusion body myositis Myopathy</td>
<td>Very high serum creatine kinase</td>
</tr>
<tr>
<td>Anti-GM1 ganglioside antibodies</td>
<td>Multifocal motor neuropathy with conduction block</td>
<td>Presence of serum anti-GM1 ganglioside antibodies</td>
</tr>
<tr>
<td>Cerebrospinal fluid analysis</td>
<td>Inflammatory neuropathy Primary progressive multiple sclerosis</td>
<td>High white cell count High protein Presence of oligoclonal bands</td>
</tr>
<tr>
<td>Nerve conduction studies (NCS)</td>
<td>Multifocal motor neuropathy with conduction block Inflammatory neuropathy Motor-predominant chronic inflammatory demyelinating polyradiculoneuropathy</td>
<td>Conduction block Demyelination</td>
</tr>
</tbody>
</table>
1.3.1. **Neuroimaging**

At present, the use of conventional neuroimaging modalities is limited to that of excluding other causes of signs and symptoms of motor neuron pathology\(^\text{20}\). While hyperintensities of the corticospinal tract\(^\text{21, 22}\) and a T2 hypointense rim in the pre-central gyrus\(^\text{22}\) may be observed on standard MRI in patients with ALS, it is not recommended to specifically search for these abnormalities in order to confirm the diagnosis\(^\text{20}\). The development of advanced MRI techniques for use in the ALS research setting is strongly encouraged\(^\text{20}\). These techniques may have a future role as diagnostic, prognostic and pharmacodynamic biomarkers serving to diagnose, stratify and monitor patients included in clinical trials.

To date, in group-level MRI studies, diffusion weighted imaging (DWI) has shown degeneration in white matter tracts including the corticospinal tract and the corpus callosum in patients with ALS\(^\text{23}\). Similar studies of grey matter changes in ALS patients found volume loss in the temporal and frontal lobes, particularly the precentral gyrus and supplementary motor cortex on T1-weighted imaging\(^\text{24}\). Grey matter metrics are suggested to be more suitable for monitoring purposes\(^\text{25}\). Extramotor involvement has been identified in ALS patients including involvement of the basal ganglia\(^\text{26}\), anterior cingulate\(^\text{27}\) and cerebellum\(^\text{28}\). In FDG-PET imaging, the most common patterns observed in ALS patients were relative hypometabolism in the frontal, medial temporal and occipital lobes and cerebellum and amygdalae\(^\text{29}\). Notwithstanding, for the purposes of serving as a diagnostic biomarker, neither advanced MRI techniques or FDG-PET are currently recommended\(^\text{20, 29}\).
1.3.2. Neurophysiology

In contrast to neuroimaging, neurophysiological data plays an important role in the diagnosis of ALS. In accordance with the Awaji electrodiagnostic criteria for ALS, neurogenic EMG changes supportive of ALS (e.g., decreased motor unit recruitment, unstable and complex motor unit potentials (MUP), MUPs of increased amplitude, duration and phase, fibrillation potentials, positive sharp waves, fasciculation potentials of complex morphology) carry the same diagnostic significance as those detected through clinical examination\textsuperscript{30}. Yet, even in the context of the increased sensitivity of these electrodiagnostic criteria\textsuperscript{31}, in patients with atypical features or who fail to progress as expected, repeated assessment is recommended in the absence of a specific diagnostic test for ALS\textsuperscript{30}. In the clinical context, EMG and nerve conduction studies also continue to play an important role in excluding alternative diagnoses\textsuperscript{30}.

Similar to neuroimaging techniques, neurophysiological approaches also offer significant potential as future diagnostic, prognostic and pharmacodynamic biomarkers. Electrophysiological approaches including EEG offer excellent temporal resolution and source resolution for less expense than traditional neuroimaging approaches\textsuperscript{32}. Resting state EEG has already been used to identify changes in brain connectivity with increased connectivity throughout the cortex found in patients with ALS\textsuperscript{33}. This is complimented by source localisation approaches which enhance the spatial resolution of connectivity measures\textsuperscript{32}. In other disorders e.g., Parkinson’s disease, EEG measures have been used to assess responses to drug therapies\textsuperscript{34}. It is hoped in the future such approaches may be adopted for the ALS population as objective quantitative outcome measures of drug efficacy for clinical trials. Transcranial magnetic stimulation (TMS) which permits the interrogation of cortical and subcortical motor networks has shown that hyperexcitability in a feature of ALS\textsuperscript{32}. TMS measures can distinguish ALS from mimic syndromes with high sensitivity and specificity\textsuperscript{35} suggesting it may have a future diagnostic role in ALS.

1.4. Clinical Management of ALS

1.4.1. Disease modifying treatments

1.4.1.1. Riluzole

Until recently, riluzole was the only available medication shown to have efficacy in slowing the progression of ALS. Following a pivotal double blind randomised controlled trial in 1994 involving 155 ALS patients that found a survival advantage and slower rate
of deterioration in muscle strength for the overall treated population\textsuperscript{36}, a subsequent larger study of 959 patients assessing drug efficacy at different doses found 100mg daily provided the best benefit-to-risk ratio\textsuperscript{37}. No difference in outcome based on site of clinical onset was found in this study despite a suggestion in the prior study that those with bulbar onset disease may benefit the most from treatment. A Cochrane review in 2012 found an overall short survival benefit of 2 to 3 months for those treated with riluzole with a 9\% increase in one year survival\textsuperscript{38}. Despite its high cost and perhaps in light of the paucity of alternative treatment options available for patients with ALS, the provision of riluzole to patients with ALS has been recommended by several bodies\textsuperscript{39,40}.

The exact mechanism of therapeutic benefit for riluzole is unknown. Initiation of riluzole treatment early in the disease course is supported by several small open label studies\textsuperscript{41,42}. However, more recent work analysing data from the original dose-ranging trial\textsuperscript{37,43} found that only those in the latest stages of disease accrued a survival benefit from riluzole\textsuperscript{43}. Riluzole is purported to work via several pathways including anti-glutamate modulation of excitotoxic pathways, promotion of neuronal survival or growth factors and modulation of Na\(^+\) and calcium-dependent K\(^+\) currents\textsuperscript{44}. Given the wide range of potential mechanisms of action offered, it is possible that riluzole may act on different therapeutic pathways at different stages of the disease\textsuperscript{45}.

1.4.1.2. Edaravone

Granted FDA approval in 2017, edaravone is the second medication licensed with a reported neuroprotective effect in ALS. Initially developed as an intravenous infusion for the treatment of acute ischaemic stroke in Japan, this free radical scavenger was subsequently adopted for use in the ALS population. Following an open label phase 2 study of 20 patients with ALS which found evidence of reduced oxidative stress in the treatment group\textsuperscript{46}, a double-blind, placebo-controlled study involving 206 ALS patients was conducted\textsuperscript{47}. No difference between treatment groups as regards primary endpoint (ALSFRS-R) was observed, however, post hoc analysis suggested a subgroup of patients was more likely to experience benefit from the compound. A subsequent phase 3, randomised, double-blind study of 137 ALS patients was conducted targeting this stringent sub-group which found a significantly smaller decline of ALSFRS-R in the treatment group\textsuperscript{48}.

Nonetheless the clinical utility of this treatment is debated with concerns over the duration of follow up, outcome measures used and lack of available biomarkers for the pivotal study\textsuperscript{49}. Subsequent studies assessing both the same stringent sub-population
and the unspecified ALS population have failed to showed efficacy for the treatment. In light of this and the potential high cost to the patient as regards time and effort spent to attend regular infusions, further studies assessing overall risk to benefit ratio for patients are recommended.

1.4.2. Symptomatic management

In the absence of curative therapies, the primary focus of clinical care in ALS is symptomatic management. The primary symptoms reported by patients with ALS are fatigue (90%), muscle stiffness (84%), muscle cramps (74%), dyspnoea (66%), sleep disturbance (60%), pain (59%), anxiety (55%), depression (52%), sialorrhea (52%), constipation (51%), pseudobulbar affect (38%), loss of appetite (37%) and weight loss (29%).

1.4.2.1. Fatigue

Fatigue is a common and incapacitating symptom without an evidence-based intervention available for its management in patients with ALS. Several drugs have been assessed for efficacy in treating fatigue in ALS patients including amantadine, pemoline and bupropion without clear benefit. Low quality evidence suggests a potential benefit for modafinil in treating fatigue compared to placebo. Similarly, there is low quality evidence supporting the use of respiratory exercises and repetitive TMS compared with sham procedures in treating ALS associated fatigue. Future larger studies assessing these three potential interventions are required.

1.4.2.2. Spasticity

Spasticity is reported as one of the most bothersome symptoms affecting most patients with ALS during the course of their disease. It is felt to reflect damage to the corticospinal tracts with reduced suprasegmental control of spinal cord reflexes and may contribute to worsening motor control, functional decline and reduced quality of life. Treatment options vary. Physiotherapy is an effective treatment choice and is recommended for all patients who experience spasticity. Baclofen, tizanidine, dantrolene and gabapentin are recommended as first line oral agents to treat spasticity in patients with ALS although the potential for side effects should be considered when starting individuals on these medications. For those not responding or tolerating these agents, intrathecal baclofen is suggested as an effective treatment option to manage spasticity associated pain and improve quality of life. One small pilot study found that injection of botulinum toxin A in individuals with ALS according to their spasticity pattern along with stretching exercises was an effective and well tolerated treatment for those with moderate to severe spasticity.
1.4.2.3. **Muscle Cramps**

Muscle cramps are a common occurrence in ALS, with no clear correlation with disease severity or duration. The cause of cramps is uncertain with both peripheral and central nervous system components proposed. Between 30-60% of patients receive some treatment directed towards this symptom despite a limited evidence base as to what treatment options are effective. The 2016 “Motor Neuron Disease assessment and management” NICE guidelines recommend quinine as a first line treatment agent. However, quinine is banned by the FDA due to safety concerns despite the absence of increased serious adverse events compared with placebo. Baclofen is recommended as a second line treatment agent for cramps with tizanidine, dantrolene and gabapentin also suggested as treatment options if baclofen is ineffective or not tolerated. A small open label pilot study suggests that levetiracetam may be beneficial in reducing cramp severity and frequency and is well tolerated. More recently, a multicentre, double-blind, placebo-controlled crossover study found that mexiletine is a well-tolerated and effectual medication for treating cramps in ALS. Carbamazepine and phenytoin have also been used in the management of cramps in ALS although there are no controlled studies to support this practice. Finally, physiotherapy or physical exercise may be useful in managing cramps in ALS although additional studies are required to assess this further.

1.4.2.4. **Pain**

While the earliest symptoms of ALS are classically characterised by painless weakness, patients with ALS may experience pain secondary to musculoskeletal strain resulting from weakened muscles. Pain may also occur as a result of spasticity and cramps (see above). Pain is managed by treating the underlying cause. Those with musculoskeletal strain may benefit from physiotherapist review, the use of supportive devices and corticosteroid injections for specific joints, where indicated. The pharmaceutical management of pain in ALS is as suggested by the WHO analgesic ladder where pain is managed initially with regular, simple analgesics, increasing in strength and adding additional classes of analgesics and adjuvants as required. The early involvement of palliative care services for those with severe pain requiring intensive palliative care interventions has been recommended.

1.4.2.5. **Sialorrhea**

Sialorrhea (or excessive saliva) results from a reduction in one’s ability to swallow secretions secondary to bulbar dysfunction. The excess of thin (serous) and/or thick (mucoid) secretions can negatively impact the lives of patients with ALS by resulting in increased fatigue, skin excoriation, compromised voice quality and social withdrawal.
There are a few studies evaluating the effectiveness and safety of the available treatment options for this symptom. Oral and topical anti-cholinergic drugs (e.g., amitriptyline, atropine, glycopyrrolate, scopolamine) are used as first line treatment agents. However, up to one third of patients do not respond to these treatments and their usage is limited by their potential for local and systemic side effects. Those with moderate to severe sialorrhea may benefit from ultrasound guided botulinum toxin injections in the parotid and submandibular glands. If effective, the beneficial effects are expected to last approximately three months. For those to fail to respond to the above therapies, radiation of the salivary glands may be used to reduce saliva production. The benefits of this treatment are expected to last for four to six months.

1.4.2.6. Pseudobulbar affect

Pseudobulbar affect is an involuntary emotional expression disorder, characterised by recurrent episodes of exaggerated or involuntary emotional expression. This disorder is common in patients with motor neuron disease, particularly in those with predominant upper motor neuron dysfunction and may result in significant embarrassment and social withdrawal. It occurs following disruption in the circuits modulating emotional initiation and output. Traditionally this disorder has been treated with off-label antidepressant medications with limited evidence supporting the use of amitriptyline and duloxetine for this indication in patients with ALS. Small studies and case reports support the use of citalopram, fluoxetine, sertraline, nortriptyline and mirtazapine to treat pseudobulbar affect in the post stroke context. A dextromethorphan-quinidine combination medication (Neudexta) was licensed in 2010 following the publication of the results of a double-blind randomised control trial, involving both ALS and MS patients, which found the treatment markedly reduced the frequency and severity of pseudobulbar affect episodes, improved quality of life and was well tolerated. In the absence of a direct head-to-head study comparing this medication with traditional antidepressant therapies, it remains unclear as to which treatment initiation strategy is preferable.

1.4.3. The Multidisciplinary Team in ALS Care

In an ideal situation, care for those with ALS should be provided through a clinic-based specialist MND multidisciplinary team approach. NICE 2016 guidelines on Motor Neurone Disease: Assessment and management recommend that the team should consist of healthcare professionals with expertise in treating patients with MND and should include a neurologist, specialist nurse, physiotherapist, occupational therapist, speech and language therapist, dietician, respiratory physiologist and a professional with expertise in palliative care which may be the neurologist or nurse. The multidisciplinary
team should offer regular assessments tailored to the patient’s symptoms and needs. The role of the multidisciplinary team is to provide information and support, address symptom management, optimise functional ability, offer psychological and social support and advise in issues pertaining to end of life care. Effective communication and coordination between all healthcare professionals involved in the patient’s care is key.

MND multidisciplinary care has been shown to improve survival for patients with ALS compared to general neurology clinics and community-based care. While it has been suggested that this may, in part, be attributable to referral centre bias in that those attending specialist clinics are more likely have younger onset disease and a longer diagnostic delay, multivariate analysis has shown that this survival advantage persists even when controlling for factors such as age of onset, diagnostic delay, site of onset, sex and riluzole, gastrostomy or NIV usage. MND multidisciplinary care has been shown to reduce both the number of unplanned hospitalisations and length of hospital stay for patients with ALS. There is limited evidence that multidisciplinary care may also improve mental wellbeing in patients with ALS but not their caregivers. The costs of multidisciplinary ALS care are in line with those of non-specialist care. The largest proportion of costs in ALS healthcare delivery arises in the community with approximately one fifth of healthcare costs being attributable to specialist multidisciplinary care. It is postulated that the advantages offered by multidisciplinary care team result from the capacity of experienced team members to engage in complex decision making, incorporating their unique perspectives into building a superior care plan for both the patient and their carer.

1.4.4. Communication and swallowing in ALS: assessment and management

Communication difficulties are common in ALS and can prevent patients from participating in various activities leading to social isolation. Dysarthria occurs in 80% of ALS patients during the course of their disease, occurring earlier in those with bulbar onset disease. Dysarthria in ALS is usually mixed spastic-flaccid type as a result of both upper and lower motor neuron involvement. It is characterised by defective articulation, nasal speech, hypophonia and disruption of prosody. Language difficulties in ALS can also lead to impairment of communication. Word retrieval, grammatical and syntactic processing and spelling deficits have all been reported in ALS. Early and regular assessments with both speech and language therapists and neuropsychologists as appropriate are recommended. The goal in managing communication difficulties in ALS patients is optimization of communication ability between the patient and their friends and family. Oral motor exercises are not recommended for speech changes in
ALS\textsuperscript{88}. Instead, strategies focusing on energy conservation are recommended\textsuperscript{89}. Appropriate timing for referral for alternative communication intervention is important as speech performance may deteriorate rapidly\textsuperscript{89}. A range of high and low technology aids are available for patients with ALS. Repeated evaluation of the suitability of different aids is imperative to allow for the expected clinical deterioration associated with disease progression\textsuperscript{89}.

Dysphagia is a presenting complaint for patients with ALS in approximately 30\% of cases. However the vast majority of patients will develop this symptom over the course of their disease\textsuperscript{90}. Dysphagia in ALS patients reflects tongue atrophy and spasticity and changes in closure of the soft palate and larynx\textsuperscript{91}. It may contribute to weight loss and dehydration in ALS patients, as well as increase their risk of aspiration\textsuperscript{92}. Regular assessment of swallowing function by an experienced speech and language therapist is recommended. Both fiberoptic endoscopic and videofluoroscopic swallowing studies may be used to evaluate swallowing function\textsuperscript{90}. The management of dysphagia is based on dietary counselling, modification of food and fluid consistencies and education of patients and carers regarding feeding and swallowing techniques\textsuperscript{52}. For those, in whom dysphagia is significantly impacting on quality of life or nutritional intake, enteral feeding approaches should be considered.

\textbf{1.4.5. Nutritional assessment and management in ALS}

Nutritional status is an important prognostic indicator in ALS\textsuperscript{93}. Patients with ALS often lose weight as the disease progresses\textsuperscript{94, 95} with marked weight loss, both pre and post diagnosis associated with more rapid progression of disease and reduced survival\textsuperscript{95-100}. There is conflicting evidence as to the prognostic utility of BMI\textsuperscript{96, 99}. Reich-Slotky et al\textsuperscript{101} suggest a non-linear relationship with an increased BMI being protective in non-obese patients but increasing risk of disease progression in obese patients. Higher survival rates were observed in those whose BMI was between 30-35 kg/m\textsuperscript{2}\textsuperscript{102}. In ALS, energy homeostasis may be significantly impaired with energy expenditure exceeding energy intake\textsuperscript{103}. Factors contributing to reduced energy intake include dysphagia, reduced appetite and difficulties in handling food\textsuperscript{96}. Furthermore, ALS patients are reported to have an approximately 10\% higher total energy expenditure compared to the healthy population\textsuperscript{104}. The exact mechanisms contributing to hypermetabolism in ALS are unknown\textsuperscript{103}.

Regular nutritional assessments by an experienced dietician should be conducted for all patients with ALS. In order to maintain their weight, patients may need advice regarding altering food consistency and modifying approaches to feeding (e.g. altered utensils,
mobile arm supports). The benefits of recommending high-calorie diets for patients with ALS remain uncertain. While there is evidence from a small phase 2 randomised placebo-controlled study that hypercaloric enteral nutrition is safe and well tolerated with a potential survival benefit for ALS patients, further studies are required to definitively examine this issue. Even more so, there is much interest from patients about the potential role of various dietary supplements with theoretical unique pharmacotherapeutic properties. Again, there is little evidence to support the use of individual agents at present. However, clinicians should be aware of the potential adverse effects of these agents and address any unrealistic beliefs with their patients.

Finally, when oral nutrition is inadequate, enteral feeding via gastrostomy may be used to support nutrition in ALS patients. The two primary methods available currently for insertion of gastrostomy are percutaneous or radiological insertion. Percutaneous endoscopic gastrostomies (PEG) are well tolerated and widely available. Radiologically inserted percutaneous gastrostomies (RIG) do not require sedation. As such they are better tolerated with higher patient satisfaction than PEG but are not as widely available. Neither method shows evidence of preventing aspiration or improved patient survival. While there is little evidence to support an optimal timing for insertion of gastrostomy, early insertion is generally recommended. The decision regarding timing of insertion of gastrostomy should be on individual patient factors including respiratory function, degree of weight loss and bulbar dysfunction and the patient’s general condition. Both EFNS and AAN guidelines recommend that gastrostomy insertion should only be considered if the patient’s FVC is greater than 50%.

1.4.6. Respiratory failure in ALS: assessment and management

Respiratory failure due to denervation weakness in respiratory muscles is the most common cause of death in ALS. Dyspnoea, orthopnoea, sleep disruption, vivid dreams, fatigue, morning headaches are all symptoms associated with respiratory failure which may negatively impact on a patient’s quality of life. Respiratory function is the best predictor of survival in ALS and repeated respiratory assessment is important for monitoring ALS progression. Different measures are available which measure different aspects of respiratory function. The measurement of vital capacity, either forced (FVC) or slow (SVC) is routine in ALS clinical care. These measure a wide variety of muscle groups involved in respiration; however, they may be impacted by non-ALS factors (e.g., other respiratory conditions, obesity etc). Measurement of vital capacity requires good facial muscle strength and good motor control which may be particularly difficult for those with bulbar onset disease. Sniff nasal inspiratory pressure (SNIP) which measures sudden rapid inhalation through the nose, preferably with one nostril
occluded, is a simple, non-invasive, inexpensive, portable test which provides a good measurement of diaphragmatic function\textsuperscript{111}. While it is easier for most patients with facial weakness to perform, it still requires good motor control. As such poor results can be difficult to interpret\textsuperscript{111}. Equally, no single test of respiratory function can reliably exclude the onset of respiratory failure\textsuperscript{109,111}.

Assisted ventilation may be used to support ventilation in ALS patients who develop respiratory failure. This can be provided either by invasive (tracheostomy) or non-invasive (non-invasive ventilation) means. Outside of Japan, where the associated costs are fully covered by the government, tracheostomy ventilation is rarely used\textsuperscript{109} due to the lack of clarity on the impact this procedure has on patients’ and caregivers’ overall quality of life\textsuperscript{112}. Non-invasive ventilation (NIV) is viewed as a more favourable alternative although prescribing practices still vary\textsuperscript{110}. NIV uses a bi-level pressure-limited ventilator to provide intermittent positive pressure support for ventilation\textsuperscript{109}. Initiation of NIV is recommended when subjective or objective evidence of daytime or nocturnal hypoventilation is present. Regular respiratory assessments should be performed to determine the most appropriate time initiate NIV\textsuperscript{113}. SNIP offers the best methods to objectively detect hypoventilation with a sensitivity of 97% for a SNIP of less than 40 cm H\textsubscript{2}O\textsuperscript{114}. Domiciliary initiation of NIV in ALS is as effective as outpatient initiation as regards patients’ respiratory function, sleep quality and quality of life but results in significantly reduced caregiver burden\textsuperscript{115}.

Several studies have found that NIV use is associated with improved survival, outperforming currently available neuroprotective therapies\textsuperscript{116}. By contrast, while 38% of patients attending an MND clinic in Ireland had access to NIV, use of NIV was not associated with increased survival. This discordant result may have been attributable to unidentified co-variants influencing adherence to NIV usage in this cohort\textsuperscript{78}. While NIV use is also associated with improved quality of life and improved cognition, the benefit appears greatest for those with orthopnoea and preserved bulbar function\textsuperscript{110,117}. Nonetheless, some uncertainties still abound over the possible unwanted effects of NIV. Further studies to assess what factors determine access to NIV, examine the health economics of NIV and explore the impact of adding cough augmentation techniques to NIV are recommended\textsuperscript{109}.

Ineffective coughing is a common and distressing symptom experienced by patients with ALS with bulbar and respiratory impairment\textsuperscript{118,119}. These patients experience an increased need to cough, complicated by a reduced capacity to do so effectively\textsuperscript{118}. As such these patients are at increased risk of developing recurrent respiratory tract infections and acute deterioration of respiratory failure\textsuperscript{119}. Effective augmentation of
cough is important to prevent the development of these complications. The best method for doing so remains debated. Two commonly used approaches include breath-stacking (using a lung volume recruitment bag) and mechanical insufflators/exsufflators (Cough Assist device). There is a lack of evidence directly comparing these two approaches. One small randomized trial comparing the two interventions found no difference in the number, duration and severity of respiratory tract infections or number of days of antibiotic usage between the two cohorts. This study was underpowered to determine group differences in survival and quality of life measures. However, in light of the lack of evidence supporting the clinical efficacy of mechanical insufflators/exsufflators, the study authors recommended adopting breath-stacking as a first line intervention due to its lower cost. Furthermore, breath-stacking has also been shown to be performed effectively by many patients with ALS with up to moderately impaired bulbar function. For those with severe bulbar involvement, therapeutic options may become limited. These patients are often intolerant of mechanical insufflators/exsufflators as they may trigger dynamic collapse of the upper airway resulting in a sensation of suffocation. Individually customized settings for pressure and flow can optimize the use of mechanical insufflators/exsufflators in this cohort.

1.5. ALS and FTD: A clinical spectrum?

Frontotemporal dementia (FTD) is a clinically and pathologically heterogeneous group of dementias characterised collectively by relatively selective, progressive atrophy involving the frontal and/or temporal lobes. It includes three clinical syndromes: (1) behavioural variant frontotemporal dementia (bvFTD) characterised by prominent behavioural change and executive dysfunction, (2) progressive non-fluent aphasia (PNFA) characterised by impaired speech production and (3) semantic dementia (SD), characterised by impaired word comprehension and semantic memory. The prevalence in populations of European origin is estimated to be between 15 to 22 per 100,000 persons. FTD typically develops in the sixth decade of life with a mean survival after diagnosis of less than 8 years. The disorder is considered to have a strong genetic basis with family history of FTD reported in approximately 50% of cases.

In recent times it has been argued that ALS and FTD can be viewed as opposite ends of a clinical spectrum, with strong clinical, pathological, radiological and genetic evidence supporting an overlap between these conditions. Despite reports of cases exhibiting both disorders dating back over 100 years, the recognition of this overlap only recently gained widespread acceptance following (1) the publication of the results of population-based neuropsychological studies in ALS cohorts and (2) the identification of novel gene mutations linking disease pathology in both disorders. The genetic and pathological
overlap between ALS and FTD will be discussed first, followed by a discussion of the clinical overlay.

### 1.6. Genetics of ALS: Introduction

The vast majority of genetically identifiable ALS is attributable to one of four gene mutations; the pathogenic C9orf72 repeat expansion and mutations in superoxide dismutase-1 (SOD1), transactive response DNA binding protein (TARDBP) and fused in sarcoma (FUS) genes. Numerous other genetic mutations have been linked to the disorder, with varying degrees of evidence supporting their pathogenicity. Known ALS associated genetic mutations are outlined in Table 1-5. While this table does not exhaust all putative ALS associated mutations, it encompasses all gene mutations showing the strongest evidence of association as well as the most comprehensively studied mutations.

### Table 1-5 ALS associated genes.

*From ALSOD\textsuperscript{132}, Zufiria et al (2016)\textsuperscript{133}, Gregory et al (2020)\textsuperscript{134}*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene name</th>
<th>Chromosome localization</th>
<th>Discovery method</th>
<th>ALSOD Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS2</td>
<td>alsin Rho guanine nucleotide exchange factor ALS2</td>
<td>2q33.1</td>
<td>L</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>ANG</td>
<td>angiogenin</td>
<td>14q11.2</td>
<td>C</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>ANXA11</td>
<td>annexin A11</td>
<td>10q22.3</td>
<td>WES</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>C9orf72</td>
<td>chromosome 9 open reading frame 72</td>
<td>9p21.2</td>
<td>L</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>C21orf2</td>
<td>chromosome 21 open reading frame 2</td>
<td>21q22.3</td>
<td>GWAS</td>
<td>Strong evidence</td>
</tr>
<tr>
<td>CCNF</td>
<td>cyclin F</td>
<td>16p13.3</td>
<td>GWAS</td>
<td>Strong evidence</td>
</tr>
<tr>
<td>CHCHD10</td>
<td>coiled-coil-helix-coiled-coil-helix domain containing 10</td>
<td>22q11.23</td>
<td>L</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>CHMP2B</td>
<td>charged multivesicular body protein 2B</td>
<td>3p11.2</td>
<td>C</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>DAO</td>
<td>D-amino acid oxidase</td>
<td>12q24.11</td>
<td>L</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>DCTN1</td>
<td>dynactin subunit 1</td>
<td>2p13.1</td>
<td>L</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Chromosome</td>
<td>Type</td>
<td>Evidence</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
<td>------------</td>
<td>-------</td>
<td>--------------</td>
</tr>
<tr>
<td>EPHA4</td>
<td>EPH receptor A4</td>
<td>2q36.1</td>
<td>F</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>ERBB4</td>
<td>erb-b2 receptor tyrosine kinase 4</td>
<td>2q34</td>
<td>L</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>EWSR1</td>
<td>EWS RNA binding protein 1</td>
<td>22q12.2</td>
<td>C</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>FIG4</td>
<td>FIG4 phosphoinositide 5-phosphatase</td>
<td>6q21</td>
<td>C</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>FUS</td>
<td>FUS RNA binding protein</td>
<td>16p11.2</td>
<td>L</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>GLE1</td>
<td>GLE1 RNA export mediator</td>
<td>9q3411</td>
<td>C</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>ERBB4</td>
<td>erb-b2 receptor tyrosine kinase 4</td>
<td>2q34</td>
<td>L</td>
<td>Moderate evidence</td>
</tr>
<tr>
<td>HNRNPA2B1</td>
<td>heterogeneous nuclear ribonucleoprotein A2/B1</td>
<td>7p15.2</td>
<td>L</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>KIF5A</td>
<td>kinesin family member 5A</td>
<td>12q13.3</td>
<td>GWAS</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>MATR3</td>
<td>matrin 3</td>
<td>5q31.2</td>
<td>L</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>NEK1</td>
<td>NIMA related kinase 1</td>
<td>4q33</td>
<td>WES</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>NEFH</td>
<td>neurofilament heavy</td>
<td>22q12.2</td>
<td>C</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>OPTN</td>
<td>optineurin</td>
<td>10p13</td>
<td>H</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>PFN1</td>
<td>profilin 1</td>
<td>17p13.2</td>
<td>WES</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>PLEK HG5</td>
<td>pleckstrin homology and RhoGEF domain containing G5</td>
<td>1p36.31</td>
<td>WES</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>SETX</td>
<td>senataxin</td>
<td>9q34.13</td>
<td>L</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>SIGMAR1</td>
<td>sigma non-opioid intracellular receptor 1</td>
<td>9p13.3</td>
<td>C</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>SOD1</td>
<td>superoxide dismutase 1</td>
<td>21q22.11</td>
<td>L</td>
<td>Definitive ALS gene</td>
</tr>
<tr>
<td>SPG11</td>
<td>SPG11 vesicle trafficking associated, spatacsin</td>
<td>15q21.1</td>
<td>L</td>
<td>Tenuous evidence</td>
</tr>
<tr>
<td>Gene</td>
<td>Description</td>
<td>Chromosome</td>
<td>Method</td>
<td>Evidence</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------</td>
<td>------------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>SQSTM1</td>
<td>Sequestosome 1</td>
<td>5q35.3</td>
<td></td>
<td>Moderate</td>
</tr>
<tr>
<td>SS18L1</td>
<td>SS18L1 subunit of BAF chromatin remodeling complex</td>
<td>20q13.33</td>
<td>WES</td>
<td>Moderate</td>
</tr>
<tr>
<td>TAF15</td>
<td>TATA-box binding protein associated factor 15</td>
<td>17q12</td>
<td>C</td>
<td>Tenuous</td>
</tr>
<tr>
<td>TARDBP</td>
<td>TAR DNA binding protein</td>
<td>1p36.22</td>
<td>L</td>
<td>Definitive ALS</td>
</tr>
<tr>
<td>TBK1</td>
<td>TANK binding kinase 1</td>
<td>12q14.2</td>
<td>WES</td>
<td>Definitive ALS</td>
</tr>
<tr>
<td>TUBA4A</td>
<td>Tubulin alpha 4a</td>
<td>2q35</td>
<td>WES</td>
<td>Strong evidence</td>
</tr>
<tr>
<td>UBQLN2</td>
<td>Ubiquilin 2</td>
<td>Xp11.21</td>
<td>L</td>
<td>Definitive ALS</td>
</tr>
<tr>
<td>UNC13A</td>
<td>Unc-13 homolog A</td>
<td>19p13.11</td>
<td>GWAS</td>
<td>Definitive ALS</td>
</tr>
<tr>
<td>VAPB</td>
<td>VAMP associated protein B and C</td>
<td>20q13.32</td>
<td>L</td>
<td>Definitive ALS</td>
</tr>
<tr>
<td>VCP</td>
<td>Valosin containing protein</td>
<td>9p13.3</td>
<td>C</td>
<td>Definitive ALS</td>
</tr>
</tbody>
</table>

ALSOD: Amyotrophic Lateral Sclerosis online Database. C: Candidate gene; F: Functional study; GWAS: Genome-wide association study; H: Homozygosity mapping; L: Linkage analysis; WES: Whole exome sequencing. ALSOD categories. ‘Definitive ALS gene’: Linkage or GWAS studies with strong functional or replication evidence; ‘Strong evidence’: well conducted recent studies, requires replication; ‘Moderate evidence’: small studies, contradictory evidence; ‘Tenuous evidence’: older small studies that have not stood up to replication.

In 1993, mutations in Cu/Zn superoxide dismutase gene (SOD1) were the first genetic cause identified for ALS\textsuperscript{135}. Since then, over 185 disease-associated variants have been reported, most of which are missense mutations. The most prevalent mutation is the D90A variant, which when homozygous is associated with slow motor progression, predominant lower limb involvement and occasional bladder disturbance\textsuperscript{136}, while in heterozygous form the variant is associated more classic ALS disease phenotype\textsuperscript{137}. In contrast, aggressive SOD1 variants including A4V, H43R, L84V, G85R N86S, and G93A are associated with more rapid disease progression and shorter survival\textsuperscript{138}. From 2008 onwards, with the discoveries of mutations in transactive response DNA binding protein (TARDBP) and fused in sarcoma (FUS) genes, the rate of genetic discoveries in ALS has increased exponentially. Carriers of TARDBP mutations usually develop a classic ALS phenotype, some exhibit parkinsonism and have a variable prognosis\textsuperscript{137},
while FUS mutations are associated with younger onset disease, marked bulbar involvement and a rapidly progressive course\textsuperscript{139}.

A significant breakthrough came in 2011, with the identification by two independent groups of pathogenic GGGGCC hexanucleotide repeat expansion in the non-coding regions of C9ORF72 gene as the cause of chromosome 9p-linked ALS and FTD\textsuperscript{140, 141}. This mutation is the most common cause of both disorders in populations of European extraction\textsuperscript{139}. From a motor viewpoint, the C9orf72 repeat expansion is associated with various phenotypes including classical ALS, PMA and PLS. A greater proportion of bulbar onset disease among carriers and a shorter median survival compared with TARDBP and SOD1 mutation carriers has also been reported\textsuperscript{142}. Interestingly, this reduced survival was shown by Rooney et al (2017)\textsuperscript{143} to be a sex-mediated effect of the variant driven by males with spinal onset disease.

Over 50% of C9orf72 carriers with ALS develop co-existent FTD\textsuperscript{144}. As this mutation is the only significant genetically identifiable cause of ALS within the Irish population\textsuperscript{145}, the neuropsychological phenotype and underlying pathology associated with the repeat expansion will be discussed separately and more extensively below. Mutations in TARDBP and FUS are responsible for a small number of ALS-FTD cases although the majority of those carrying these mutations present with a classical ALS phenotype without cognitive impairment\textsuperscript{137}. By contrast, cognitive impairment is very rare in carriers of SOD1 mutations, occurring in less than 3% of those presenting with ALS\textsuperscript{144, 146}. Rarer ALS associated genetic mutations including ANG, CHMP2B, DCTN1, GRN, hnRNPA1, MATR3, OPN, UBQLN2 and VCP have also been associated with FTD phenotype\textsuperscript{137}.

In the FTD population, other than the C9orf72 repeat expansion, mutations in two other genes account for majority of cases in whom a genetic cause is identified: microtubule-associated protein tau (MAPT) and granulin (GRN). MAPT was the first gene associated with FTD and proved that tau dysfunction without amyloid pathology was sufficient to cause neurodegeneration\textsuperscript{147}. Mutations in the gene account for 20% of familial FTD cases\textsuperscript{148} and is associated with young onset disease, early amnesia, personality and behavioural disturbance and atypical parkinsonism\textsuperscript{149}. GRN mutations account for 5 to 20% of familial FTD cases and usually presents with a behavioural variant FTD presentation, with late extrapyramidal signs. Visual hallucinations, amnesia and parietal dysfunction may also occur\textsuperscript{147}. Tenuous evidence at best links GRN and TARDBP mutations to ALS, including case reports describing the detection of mutations in these genes in patients with ALS-FTD\textsuperscript{150, 151} and ALS without cognitive impairment\textsuperscript{152}.
1.7. Neuropathology

The gross pathological features of ALS primarily consist of atrophy of the motor cortex, pyramidal tracts and skeletal muscle. Axonal degeneration of the corticospinal and corticobulbar motor neurons results in the characteristic scarred appearance of the lateral spinal cord ("lateral sclerosis") while degeneration of bulbar or spinal motor neurons leads to denervation and atrophy of their target muscles ("amyotrophy")\textsuperscript{153, 154}. In FTD cases, similar but much more extensive frontal and temporal lobe atrophy is seen, with bvFTD cases showing early bilateral orbito-mesial and dorsolateral frontal cortex atrophy followed by temporal lobe and basal ganglia degeneration\textsuperscript{155}. At a microscopic level, in ALS and FTD, reactive astrocytes and microglia, and other signs of neuroinflammation, often accompany the progressive loss of motor neurons\textsuperscript{156}.

The neuropathological hallmark of the condition may be found in the surviving neurons which often contain ubiquitinated cytoplasmic protein aggregates\textsuperscript{156}. The identification of TDP-43 (trans-activating responsive DNA-binding protein) as the major ubiquitinated protein found in sporadic ALS patients and half of all FTD patients was a key breakthrough in linking both disorders\textsuperscript{157, 158}. While these protein aggregates account for the vast majority of ALS cases, SOD1, FUS and other protein aggregates have been detected in a smaller percentage of cases \textsuperscript{157}. In FTD, an equal proportion of the cytoplasmic protein aggregates are attributable to hyperphosphorylated tau as TDP-43\textsuperscript{159} (Figure 1-1). The abnormal intracellular accumulation of the former is seen in several FTD associated disorders including Pick’s disease, corticobasal degeneration and progressive supranuclear palsy (tauopathies). In contrast to ALS, where FUS aggregates occur in isolation\textsuperscript{160}, 5-10\% of FTD cases are characterized by intracellular aggregates of FUS, Ewing’s sarcoma (EWS) and TATA-binding protein associated factor 15 (TAF15) which are broadly designated under the term FTLD-FET\textsuperscript{161}. As such, it is now popular to subdivide FTLD pathology into broad categories based on predominant protein aggregate (FTLD-tau, FTLD-TDP, FTLD-FET and FTLD-UPS [ubiquitin proteasome])\textsuperscript{161}. 
What role cytoplasmic protein aggregates play in ALS and FTD disease pathophysiology is not entirely clear. In ALS, multiple pathogenic mechanisms have been proposed including (1) impaired protein homeostasis, (2) aberrant RNA metabolism, (3) impairments in nucleocytoplasmic, endosomal and vesicle transport, (4) cytoskeletal and axon-transport defects, (5) impaired DNA repair, (6) excitotoxicity, (7) oligodendrocyte degeneration, (8) neuroinflammation and (9) mitochondrial dysfunction. Thematic analysis of the common pathways affected by multiple ALS causative gene mutations, suggest that of the above aberrant RNA processing and protein degradation may be the most important disease mechanisms (Figure 1-2). These are discussed in more detail below.

### 1.7.1. Aberrant RNA processing

Substantial focus on the role of RNA biology in ALS pathogenesis has stemmed from the recognition of the importance of RNA-binding proteins in earliest stages of disease development. Both TDP-43 and FUS are heterogeneous nuclear ribonucleoproteins (hnRNPs), a family of RNA-binding proteins that regulate RNA metabolism.
Cytoplasmic mis-localization of TDP-43 or FUS is can be seen in affected neurons of most ALS patients, as well as in a notable proportion of FTD patients. Mutations in the low complexity sequence domains (LCDs) of RNA-binding proteins such as TDP-43 and FUS\textsuperscript{162, 163} may mediate phase separation resulting in the formation of pathological amyloid-like fibrils in cell bodies\textsuperscript{164}. These, in addition to other mechanisms such as poorly dynamic cytoplasmic RNA granules and defects in nucleocytoplasmic trafficking, may contribute to the redistribution of TDP-43 and FUS from the nucleus to the cytoplasm\textsuperscript{154, 157}. Depletion of RNA-binding proteins from the nucleus can cause a relative loss of nuclear function resulting in altered regulation of alternative splicing and depletion of RNA coding synaptic proteins and correctly spliced protein encoding mRNAs\textsuperscript{165, 166}. Furthermore, loss of TDP-43 or FUS can result in impaired biogenesis of microRNAs which are necessary for the maintenance of neuromuscular junctions\textsuperscript{153, 154}.

1.7.2. Aberrant protein degradation

Impaired protein degradation by means of disruption of the ubiquitin-proteasome and autophagy systems is proposed as a central disease mechanism in ALS and FTD\textsuperscript{154}. Mutations in SOD1, VCP and UBQLN2 have been associated with impaired expression of components of the ubiquitin-proteasome system and altered substrate delivery to the proteasome\textsuperscript{153}. Defective autophagy could also partially explain the toxic accumulation of both TDP-43 and SOD1 as these are known substrates of autophagy, as is C9orf72\textsuperscript{153}. Interestingly, TDP-43 and FUS are involved in both aberrant RNA processing and protein degradation suggesting a central role for these processes in ALS pathogenesis\textsuperscript{156}. Indeed, both processes are interconnected. Aberrant RNA processing can impair protein degradation as the secondary TDP-43 depletion in aggregates can inhibit autophagy\textsuperscript{156}. Conversely, impaired protein degradation can result in aberrant RNA processing through TDP-43/FUS aggregation in stress granules, which sequester mRNA and RNA-binding proteins\textsuperscript{156}.
1.7.3. C9orf72 associated neuropathology

The pathogenic C9orf72 repeat expansion is proposed to work through three non-exclusive pathogenic mechanisms in ALS and FTD (Figure 1-3). Repeat expansion sense and antisense transcript expression suggests a toxic gain of function through two primary mechanisms: (1) sequestration of RNA-binding proteins and (2) abnormal translation resulting in the production of potentially toxic dipeptide repeat proteins (DPR).

Both sense and antisense containing RNA foci have been shown to accumulate in affected cells. In other triplet repeat disorders, the accumulated repeat-containing RNA results in sequestering of RNA-binding proteins with subsequent impaired RNA splicing. C9orf72 models have shown impaired RNA transport and function secondary to sequestration of some hexanucleotide repeat-binding proteins, but to what degree this sequestering may contribute to the disease pathology remains unknown. Toxic gain of function is also purported to occur due to abnormal repeat associated RNA-encoded (RAN), non-ATG translation resulting in the production of several potentially toxic dipeptide repeat proteins. The detection of these proteins in TDP-43-negative neuronal
inclusions in ALS and FTD supports their pathogenicity, however discrepancies in the correlation between the burden of DPR deposition and severity of neurodegeneration need further study to determine their exact role in disease pathology.

Loss of function of the gene containing the repeat (haploinsufficiency) is associated with a modest reduced mRNA levels but not necessarily C9orf72 proteins. Overall, the dominance inheritance patterns of C9orf72 positive ALS and FTD, the dearth of null alleles or missense mutations in C9orf72 in ALS and FTD patients and the paucity of neurodegeneration in C9orf72 knockout mice suggest that haploinsufficiency in itself may be insufficient to cause disease but may impact on the disease phenotype.

Figure 1-3: Three non-exclusive mechanisms have been proposed for C9orf72 associated ALS/FTD.

From Taylor et al (2016).

1.8. Neural networks in ALS

Two principal models of how neurodegeneration develops in ALS have been proposed – the ‘dying backwards’ hypothesis where ALS is primarily a disease of the lower motor neurons spreading transsynaptically to the upper motor neurons, primarily monosynaptically. In contrast the ‘dying forward’ hypothesis proposes that ALS is a disorder where neuronal degeneration originates in the motor neurons of the cortex), spreading to the anterior horn cells. While this corticofugal model of neurodegeneration...
was first proposed by Charcot in his original descriptions of ALS, it received little attention until revived in a review by Eisen and Weber (2001). In the interim, proponents of the ‘dying backwards’ hypothesis found support for their model in pathological studies demonstrating early axonal dysfunction preceding motor neuronal degeneration and a greater degree of LMN loss than UMN loss, suggesting primary rather than secondary LMN involvement. Further evidence supporting this hypothesis includes the clinical and pathological overlap between PMA and ALS with some patients initially diagnosed as PMA going on to develop classical ALS.

However, a similar argument may be made by proponents of the ‘dying forwards’ hypothesis, as some patients with PLS may continue on to develop ALS. Those supporting the ‘dying forward’ hypothesis point to evidence of multisystem involvement in ALS. Furthermore, dissociated muscle wasting patterns including the split-hand phenomenon, a specific clinical feature of ALS, reflect cortical dysfunction as these fractionated movements are executed by the primary motor cortex. These clinical arguments are supported by imaging and neurophysiological findings. Cortical abnormalities in ALS, including extra-motor involvement, have been observed, which are not compatible with the ‘dying backwards’ hypothesis. Similarly, cortical hyperexcitability has been observed in ALS patients as evidenced by reduced short interval intracortical inhibition (SICI) and cortical silent period (CSP) reflecting inhibitory cortical interneuron dysfunction. Together, the bulk of the evidence now supports the concept of ALS as a disorder of specific neural networks, similar to what is seen in other neurodegenerative disorders.

Neural networks are defined as a complex series of interconnected neural nodes representing neurons and their connections and can be considered at (1) microscopic or macroscopic levels (neurons and synapse or anatomical regions and tracts), (2) structural or functional (physical versus physiological connections) or by (3) the predominant pathogenic protein involved. For example, the salience network comprises the dorsal anterior cingulate cortex and frontoinsular cortex with links to the superior temporal pole, dorsolateral prefrontal cortex, amygdala, thalamus, hypothalamus and the substantia nigra/ventral tegmental area and is responsible for detecting, analysing and integrating emotionally salient stimuli with one’s internal environment. Abnormalities of this network explain the profound disinhibited behaviours and reduced empathy seen in ALS and FTD, which are described in more detail below.
1.9. **Cognitive and behavioural changes in ALS**

Several population-based studies have now confirmed that approximately 15% of those diagnosed with ALS, will also meet criteria for FTD during their disease\textsuperscript{16, 177}. Furthermore, approximately 40% and 30% of those with ALS will experience milder forms of cognitive and behavioural impairment respectively\textsuperscript{16, 176}. In contrast, approximately 13% of those with FTD have concomitant ALS with an additional 30% exhibiting mild motor dysfunction such as fasciculations or mild wasting and weakness\textsuperscript{178}. Executive function is the most common cognitive domain impaired in patients with ALS, with language dysfunction occurring independently of executive dysfunction increasingly recognised\textsuperscript{87}. Older age, male sex, bulbar onset disease and lower educational attainment have been identified as risk factors for cognitive involvement in ALS, but these associations have not been reported consistently\textsuperscript{179}.

Executive difficulties observed in ALS populations include deficits in verbal fluency, attention and working memory and difficulties in reasoning, coordinating rules and mental heuristics\textsuperscript{180}. Impaired letter fluency has been found to occur at an early disease stage with letter fluency shows greater impairment in ALS patients than categorical fluency\textsuperscript{180, 181}. Tasks such as the KDEFS sorting test, reverse digit span, digit recall, colour-word interference test, the medication scheduling task and holiday apartment task may be used to assess for evidence of executive dysfunction in this population. Compared with executive function, the study of language involvement in ALS has been relatively under-investigated\textsuperscript{182}. The verbal fluency deficits observed in ALS population can also potentially arise as result of impaired lexical access\textsuperscript{87}. Grammatical and syntactic processing deficits, independent of executive dysfunction, have been recorded in ALS populations, as have spelling deficits\textsuperscript{87}. Tasks such as the those from the Psycholinguistic Assessments of Language Processing in Aphasia (PALPA) battery as well as the Boston naming test and pyramids and palm tree test have been used to assess cognition in ALS\textsuperscript{177}. Neuroimaging studies have observed frontal lobe changes including in the dorsolateral prefrontal cortex, orbitomedial prefrontal cortex and inferior frontal gyrus in ALS patients with executive dysfunction. Similarly, evidence of neuroanatomical involvement in ALS in language centers including the posterior, inferior frontal, the posterior inferior parietal and superior temporal areas correlating with deficits in verbal fluency, grammatical and syntactic processing and spelling has also been observed\textsuperscript{87}.

More recently, deficits in social cognition have been recognised as central to the cognitive profile associated with ALS. Whether these deficits occur independently of
executive dysfunction or not remains a matter of debate\textsuperscript{183,184}. Patients with ALS have shown impaired capacity to recognise emotional facial expressions and have difficulties with Theory of Mind tests\textsuperscript{180}. Such deficits do not necessarily imply direct orbitofrontal cortex involvement but could also be mediated by dysfunction of other regions including the dorsolateral and medial prefrontal and limbic systems which link to this region\textsuperscript{185}. Such social cognition deficits are near ubiquitous in patients with ALS-FTD\textsuperscript{180}. In contrast memory and visuospatial deficits are rare in ALS. The co-existence of Alzheimer’s disease in patients with ALS occurs in approximately 2% of cases\textsuperscript{16}, in line with the prevalence expected for the general population\textsuperscript{186}. While memory deficits are not considered sufficient to meet criteria for a diagnosis of ALS with cognitive impairment\textsuperscript{180}, variable difficulties including impaired encoding and immediate or delayed recall have been reported in the ALS population\textsuperscript{187,188}. The latter deficits were found to correlate with grey matter hippocampal volumes in ALS patients\textsuperscript{189}. Nonetheless, difficulty arises in distinguishing whether these deficits are the primary insult or occur secondary to executive dysfunction. In one population-based study of cognition in ALS patients, after accounting for executive impairment, the frequency of memory deficits in ALS patients was not significantly increased compared with controls\textsuperscript{16}.

Mild to moderate behavioural changes are estimated to occur in approximately 30% of patients with ALS, with an additional 13% of patients having severe behavioural impairment meeting criteria for bvFTD\textsuperscript{176}. Apathy is the most common behavioural symptom identified in ALS patients, correlating with nucleus accumbens atrophy\textsuperscript{180}. Other behavioural changes observed include disinhibition, impulsivity, irritability, emotional lability, loss of sympathy, new unusual habits, perseverative and stereotyped behaviour and changes in dietary habits and hygiene\textsuperscript{176,180}. One cross-sectional population-based study assessing behaviour change in ALS identified five behavioural sub-phenotypes using factor analysis. These sub-phenotypes included those with primarily 1) disinhibited behaviour reflecting disruption of the medial prefrontal and orbitofrontal cortices and the anterior insula, 2) impaired impulse control reflecting disruption of the orbitofrontal and anterior cingulate pathways, 3) dysexecutive behaviours reflecting involvement of the right prefrontal regions, 4) cognitive rigidity reflecting dorsolateral prefrontal cortex and temporolimbic networks involvement and 5) severe behavioural change meeting criteria for ALS-FTD and neuropsychiatric symptoms. The fifth sub-phenotype was not predictive of any specific neuroanatomical pathways but rather was driven by those carrying the C9orf72 repeat expansion\textsuperscript{176}. Behavioural changes were qualitatively similar for those with mild versus severe impairment suggesting more severe impairment reflects greater disruption of the affected
neural pathways. Furthermore, while behavioural change may be mediated by deficits in executive and social cognition function, behavioural changes was also found to occur independent of cognitive changes. Behavioural impairment among patients was found to be a major determinant of caregiver burden in ALS, more important than cognitive or physical impairment.

Whether or not, cognitive and behavioural changes progress in line with motor deterioration is yet undetermined. Longitudinal studies assessing cognition in ALS populations are limited by clinic-based populations, small numbers and high attrition rates. Indeed, those with cognitive impairment at baseline have higher attrition rates meaning that the overall cognitive decline of the study cohorts may be underestimated. Nonetheless, one population-based longitudinal study of cognition in an Irish ALS population found that normal cognitive function at baseline was associated with a propensity to remain cognitively well, while those with cognitive impairment at baseline tended towards faster cognitive decline. Taking disease stage, rather than time, as a measure of motor progression, Crockford et al (2018) found that a greater burden of ALS-specific cognitive and behavioural impairment in those with more advanced stage disease suggesting that these domains should be incorporated into future staging systems.

Nonetheless, while it is now widely accepted that cognitive and behavioural changes are associated with ALS, it is worth commenting on the limitations of the available literature. There is marked variability in the specific tasks used to assess neuropsychological function in ALS, limiting capacity to perform substantive meta-analyses of the data. This is further complicated by need in many cases to modify tasks (and the variability in approaches in this regard) to address the accrued physical disabilities in patients. In many neuroimaging studies, the neuropsychological characterisation of patients is a secondary objective, if performed at all, ensuing in a missed opportunity to connect neuropsychological dysfunction with specific anatomical changes. Furthermore, concerns can be raised as to the generalizability of neuropsychological findings to the broader ALS cohort because of the potential confounding by sources of referral biases. Patients attending specialised ALS clinics may be more likely to manifest atypical or more severe cognitive or behavioural dysfunction than those attending general neurology clinics. As such, studies of clinic-based ALS populations may overestimate the severity and frequency of neuropsychological dysfunction. Despite, and in part because of, these limitations the neuropsychological characterisation of ALS patients...
remains an interesting area for further studies which may provide insights into the nature of network disturbance in the condition.

1.9.1. Classification criteria

Two classification criteria are worth consideration with regard to the cognitive and behavioural changes observed among ALS patients. The first are criteria proposed by the International Behavioural Variant FTD Criteria Consortium (FTDC) for the diagnosis of behavioural variant FTD\(^{196}\). These build on previous guidelines proposed by Neary et al (1998)\(^{197}\) but offer greater sensitivity for disease diagnosis with less restrictive exclusion criteria. The second criteria are those proposed by Strong et al (2017)\(^{180}\) for the purpose of classifying milder neuropsychological changes associated with ALS. These criteria expand on a previously published version\(^ {198}\) to include both language impairment as evidence of cognitive impairment in ALS and primary progressive aphasia as a sub-category of ALS-FTD. Both classification criteria are outlined in the tables below (Table 1-6; Table 1-7). Strong criteria refer to the second axis of their guideline, specifically describing diagnostic criteria for cognitive and behavioural syndromes in ALS.

Table 1-6: International Consensus Criteria for behavioural variant FTD (bvFTD).

*From Rascovsky et al (2011)\(^ {196}\).*

<table>
<thead>
<tr>
<th>1. Neurodegenerative disease (the following symptom must be present)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progressive deterioration of behaviour and/or cognition by observation or history(^ a )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Possible bvFTD: Three of the following behavioural/cognitive symptoms(^ b, c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Early behavioural disinhibition</td>
</tr>
<tr>
<td>b Early apathy or inertia</td>
</tr>
<tr>
<td>c Early loss of sympathy or empathy</td>
</tr>
<tr>
<td>d Early perseverative, stereotyped or compulsive/ritualistic behaviour</td>
</tr>
<tr>
<td>e Hyperorality and dietary changes</td>
</tr>
<tr>
<td>f Neuropsychological profile: executive/generation deficits with relative sparing of memory and visuospatial functions</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Probable bvFTD: Meets criteria for ‘Possible’ bvFTD plus both</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Significant functional decline</td>
</tr>
<tr>
<td>b Imaging results consistent with bvFTD (MRI and/or PET/SPECT)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Behavioural variant FTD with definite FTLD Pathology: Probable bvFTD plus either</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Histopathological evidence of FTLD on biopsy or at post-mortem</td>
</tr>
<tr>
<td>b Presence of a known pathogenic mutation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5. Exclusionary criteria for bvFTD</th>
</tr>
</thead>
</table>
- Pattern of deficits is better accounted for by other non-degenerative nervous system or medical disorders
- Behavioural disturbance is better accounted for by a psychiatric diagnosis
- Biomarkers strongly indicative of Alzheimer’s disease or other neurodegenerative process

A. information provided by a knowledgeable informant. B. Symptoms must be persistent or recurrent. C. ‘Early’ refers to symptom presentation within the first 3 years.

**Table 1-7: Diagnostic classification of frontotemporal syndromes in ALS.**

*From Strong et al. (2017)*

<table>
<thead>
<tr>
<th>ALS with behavioural impairment (ALSbi)</th>
<th><strong>diagnosis requires either</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Identification of apathy with or without other behaviour change</td>
</tr>
<tr>
<td>b</td>
<td>Meeting at least two non-overlapping supportive diagnostic features from the Rascovksky criteria</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ALS with cognitive impairment (ALSci)</th>
<th><strong>requires evidence of either/both</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Executive dysfunction, including social cognition (requires either)</td>
</tr>
<tr>
<td>i</td>
<td>Impaired verbal fluency (letter)</td>
</tr>
<tr>
<td>ii</td>
<td>Impairment on two other non-overlapping measures of executive functions (which may include social cognition)</td>
</tr>
<tr>
<td>b</td>
<td>Language dysfunction</td>
</tr>
<tr>
<td>i</td>
<td>Impairment on two non-overlapping tests and in which language impairment is not solely explained by verbal fluency deficits</td>
</tr>
</tbody>
</table>

**ALS with combined cognitive and behavioural impairment (ALS-cbi)**

Meet the criteria for both ALSci and ALSbi

**ALS-FTD**

**requires both**

| a                          | Evidence of progressive deterioration of behaviour and/or cognition by observation or history |
| b                          | Any of |
| i                          | The presence of at least 3 of the behavioural/cognitive symptoms outlined by Rascovksky criteria |
| ii                         | The presence of at least 2 of those behavioural/cognitive symptoms, together with loss of insight and/or psychotic symptoms |
| iii                         | The presence of language impairment meeting criteria for semantic dementia/semantic variant PPA or non-fluent variant PPA.

A. May co-exist with behavioural/cognitive symptoms outlined.
1.9.2. Neuropsychological and neuropsychiatric changes in C9orf72 carriers

Studies assessing the cognitive, behavioural and neuropsychiatric characteristics of C9orf72 repeat expansion carriers have to date focused primarily on cohorts of patients with FTD, with or without co-existent ALS. Even among studies of ALS cohorts, over 50% of carriers had co-existent FTD at time of diagnosis\textsuperscript{144, 177}. As such, there is little in the available literature describing the earliest cognitive, behavioural and neuropsychiatric changes observable in C9orf72 carriers. The neuropsychological characteristics of C9orf72 carriers, described below, reflect the findings in those with more advanced disease, with significant overlap with features seen in FTD without known genetic cause.

Most C9orf72 positive FTD patients present with behavioural variant form of FTD, although this is also true for all FTD patients in general. A study by Irwin et al (2013)\textsuperscript{199} did not find any difference in the proportion of C9orf72 positive FTD patients presenting with behavioural variant compared to patients with FTD for whom a genetic cause has not been identified. Executive impairment is pervasive among C9orf72 carriers with FTD. Deficits in C9orf72 positive FTD patients have been observed with Block Design, Trial Making Test Part B, Stroop colour-word interference test and verbal fluency tasks\textsuperscript{200}. Executive impairment is frequently present at the baseline assessment, and in some studies was shown to progress with time\textsuperscript{199, 201}. Language impairment is also relatively common in C9orf72 carriers with FTD. Kaivorinne et (2013)\textsuperscript{202} reported on speech and language difficulties in 84% of C9orf72 positive FTD cases recruited through a specialist memory clinic. While the authors did not distinguish between speech difficulties attributable to ALS-associated dysarthria and there is an inherent referral bias in that specialist clinics are more likely to see atypical clinical presentations, it is notable nonetheless the observation that 6 out of 22 C9orf72 carriers had progressive non-fluent aphasia (PNFA), including two expansion carriers with ALS-FTD. While Snowden et al (2012)\textsuperscript{203} reported word finding difficulties in a small C9orf72 positive FTD cohort suggesting semantic language impairment, this was not found to be significantly different compared to matched non-carriers in a follow up study by this group\textsuperscript{204}. Memory impairment is also a relatively common finding in C9orf72 carriers with FTD, occurring in up to 40% of patients, but often associated with impairment on executive function tasks\textsuperscript{205}. Similarly, visuospatial deficits observed in C9orf72 carriers, while rare, are usually identified on construction tasks requiring greater executive processing\textsuperscript{206}. As such, it remains uncertain at this time whether memory and visuospatial networks are
independently impaired in C9orf72 carriers, or whether changes observed arise secondary to executive dysfunction.

Disinhibition and apathy are the most common behavioural changes observed among C9orf72 carriers with FTD and ALSFTD. The prevalence of disinhibitive symptoms varies between 13% to 76%\textsuperscript{201}, with apathy reported to occur in 30 to 63% of carriers\textsuperscript{200}. Apathy without disinhibition among C9orf72 carriers is infrequent, occurring in just 14% of carriers with FTD\textsuperscript{207}. C9orf72 carriers with FTD shows less dietary changes compared with matched non-carriers\textsuperscript{203}. This is attributed to a reduced preference for sweet food among C9orf72 carriers with FTD, with no difference compared to matched non-carriers observed for overeating/ food cramming behaviours\textsuperscript{203}. Furthermore, stereotyped/repetitive behaviours were relatively common in C9orf72 carriers with FTD (prevalence 15% to 25%)\textsuperscript{201, 202, 208}, often characterised by complex routines, which at times have an obsessional quality\textsuperscript{203}. While disinhibition, apathy and repetitive behaviour are core features of FTD, and there is conflicting evidence as to whether these symptoms occur at a higher frequency in C9orf72 carriers than non-carriers\textsuperscript{204, 209}, it is interesting that in a review by Boeve et al (2012)\textsuperscript{210} the authors noted that the behaviour changes observed in C9orf72 positive FTD patients were often “the most bizarre behaviours manifestations” the treating physicians had ever encountered.

Finally, psychotic symptoms, including delusions and hallucinations, are common in C9orf72 positive FTD and ALSFTD, although it remains unclear as to whether they occur more frequently than in the general FTD or ALS-FTD population\textsuperscript{204, 209, 211}. 5 to 77% of carriers with FTD manifest psychotic features during the course of their disease\textsuperscript{200, 203, 208}, with those presenting initially with psychotic features more likely to carry the repeat expansion\textsuperscript{203}. Delusions and hallucinations are the most common psychotic symptoms associated with the C9orf72 repeat expansion, with a prevalence among carriers of up to 50% in some studies\textsuperscript{200, 212}. The delusions observed in C9orf72 carriers with FTD are usually of the paranoid type\textsuperscript{212}, are sometimes somatic and may fail to respond to anti-psychotic medication\textsuperscript{203}. A small study by Sha et al (2012)\textsuperscript{209} examining 15 C9orf72 positive FTD cases noted that reports of delusions as an initial neuropsychiatric symptom distinguished C9orf72 positive cases from matched non-carriers. However, no difference in delusions between carriers and matched non-carrier was observed at first evaluation using the Neuropsychiatric Inventory, suggesting that delusions may only be specific to C9orf72 repeat expansion if reported as the first symptom\textsuperscript{212}. Both visual and auditory hallucinations have been reported in C9orf72 carriers with FTD\textsuperscript{203, 213}, and are often complex in nature\textsuperscript{203}. A review by Takada and Sha (2012)\textsuperscript{212} reported that hallucinations
are more likely to occur later in the disease course. Yet, reports of C9orf72 carriers with FTD developing hallucinations up to 20 years before the onset of typical FTD symptoms are also noted\textsuperscript{213}. Several cases of overt obsessive-compulsive disorder in C9orf72 carriers with FTD and ALSFTD have also been reported\textsuperscript{144, 208}. Finally, depression and anxiety are relatively frequent among FTD and ALSFTD cases whom carry the repeat expansion, with estimated prevalence of between 10-20\%\textsuperscript{212} and 30-50\%\textsuperscript{201} respectively.

Many of the limitations of neuropsychological studies, discussed above, apply to an even greater degree in studies assessing C9orf72 carriers. The rarity of the mutation makes recruiting a sufficient population to characterise difficult. The variability of the neuropsychological assessments which often overlap in the domains they assess, makes it challenging to determine the exact networks impaired by the C9orf72 repeat expansion. Increasing recognition of the neuropsychological manifestations of C9orf72 associated FTD also poses a further challenge. A pattern of (1) relative sparing of memory and visuospatial functions in those with executive dysfunction and (2) exclusion of underlying psychiatric conditions are both requirements in the current International Consensus Diagnostic criteria for FTD\textsuperscript{196}. As such, the unique cognitive and neuropsychiatric profile of C9orf72 carriers can often render diagnosing FTD problematic, an observation highlighted in a study by Devenney et al (2014)\textsuperscript{211} of 10 C9orf72 carriers with clinical FTD, of whom most did not meet the formal consensus criteria for the diagnosis.

1.10. Disease progression and staging in ALS

Disease staging criteria offer the opportunity to monitor disease progression in respect to defined clinical milestones chosen to reflect disease severity and prognosis\textsuperscript{214, 215}. In contrast, measures such as the ALSFRS-R rating scale assess functional decline independently of such milestones. While the slope of the change in ALSFRS-R score has important prognostic implications, there no clear threshold at which a change in ALSFRS score is felt to reflect an important transition point in functional status\textsuperscript{214}. Assessing disease progression in relation to defined clinical milestones is more intuitive for both physicians, patients and their carers to understand the disease and its clinical course better\textsuperscript{214}. In different stages of the disease patients will have different needs with earlier stages requiring diagnostics and various therapeutic supports, whilst later stages necessitate respiratory and nutritional interventions and a more palliative approach to care\textsuperscript{214-216}. Staging criteria help guide therapeutic decision making, resource allocation and patient selection and outcome assessment in clinical trials\textsuperscript{214, 215}. ALS-specific
staging systems are shown to correlate with functional measures, quality of life scores, disease biomarkers, health utility and healthcare and socioeconomic costs.

There are two available staging systems used in ALS to measure disease progression: The King’s staging system and the Milano–Torino (MiToS) Staging system. The King’s staging system has five different stages, from 1 to 5 and is based on neuroanatomical distribution of the disease and disease burden as measured by significant nutritional or respiratory failure. The MiToS system has six stages, from 0 to 5 and is established from one’s functional ability as measured by the ALSFRS-R. In both systems, stage 5 equates with death. A retrospective study comparing both staging systems found greater resolution in early to mid-disease with the King’s staging system compared with higher resolution in late disease for the MiToS staging system. This reflects the necessity that functional decline stems from anatomical involvement.

Both systems are limited by their failure to include a measure of cognitive involvement, a known negative prognostic indicator which necessitates its own specific management approach. As cognitive impairment may develop at any time in the disease course, it is therefore difficult to translate cognitive involvement to a clear clinical milestone within an expected temporal sequence. While future ALS staging systems may include some measure of cognitive and/or behavioural impairment, at present the use of criteria grading the severity of cognitive and behavioural impairment in ALS is recommended in parallel to current ALS disease staging systems.

1.10.1. Prognosis

ALS is often referred to as the “1000-day disease” as most people with this condition are expected to die within this time period following symptom onset. The median survival from onset to death is reported as ranging from between 20 to 48 months, although this range is decreased when only population-based studies are assessed (20-36 months). Approximately 5 to 10% of patients are expected to survive for longer than 10 years. Individual survival and rate of disease progression varies significantly. Factors that are associated with a worse prognosis include increased age at symptom onset, bulbar and respiratory onset disease, definite ALS according to the revised El Escorial criteria at first presentation, lower predicted forced vital capacity (FVC%) at diagnosis, weight loss, cognitive dysfunction, faster progression rate and specific gene mutations e.g. C9orf72 repeat expansion. By contrast, factors that are reported to be associated with a
more favourable prognosis include longer delay from symptom onset to diagnosis, specific gene mutations e.g., certain SOD1 mutations, and riluzole usage\textsuperscript{219}.

Recently, a personalised prognosis prediction model for ALS has been developed and validated by members of the ENCALS consortium which is hoped will aid in individualised patient management, counselling and future clinical trial design\textsuperscript{221}. 8 out of 16 candidate predictors were selected for the multivariable prediction model including bulbar onset disease, age at onset, definite ALS according to the revised El Escorial criteria, diagnostic delay, FVC, progression rate, frontotemporal dementia and the presence of the C9orf72 repeat expansion. This prediction model provides accurate predictions at an individualised level allowing for the prognostic categorisation of patients with ALS into five distinct groups (very short, short, intermediate, long and very long with median predicted survival from symptom onset in months of 18, 25, 32, 44 and 91 months respectively). This model has allowed for the retrospective examination of Stephen Hawking’s prolonged survival which was found to be in keeping with current knowledge of disease progression\textsuperscript{222}.

1.11. **ALS Epidemiology**

ALS is a rare disease with an estimated incidence of between 0.6 and 3.8 per 100,000 person-years and prevalence of between 4.1 and 8.4 per 100,000 persons\textsuperscript{223}. The incidence and prevalence of ALS varies widely with geographical region, with notably high incidence reported in some small isolated populations. Clusters of ALS and Parkinsonism-dementia complex (PDC) were observed Guam, Kiji peninsula in Japan and New Guinea during the mid to late 20\textsuperscript{th} century, with peak prevalences of over 50 per 100,000 persons\textsuperscript{224}. While the rapidly decreasing incidence of the disease in these regions favours the environmental cause hypothesis, in that changing lifestyles may have resulted in the removal of an unknown exposure, the observation that the disease developed within isolated ethnic populations in these regions suggests that these populations may have been genetically predisposed to some degree. In the Faroe Islands, the high incidence seen may reflect excellent case ascertainment in a small population\textsuperscript{225}, but similarly could also be attributable to genetic founder effect shared with other Scandinavian populations, compounded by increased genetic homogeneity in the population due to its isolated location.

In contrast much lower ALS incidence rates are reported among some Asian and African populations, with the lowest incidence in East Asia of 0.89, South Asia of 0.79 and South Africa of 1.1 per 100,000 person years respectively\textsuperscript{224, 226}. The lower incidence observed in these regions may reflect under-ascertainment of cases, differences in study design.
or true demographic/geographic differences. Regarding the former, Chio et al. (2013) recommend all epidemiological studies should be prospective, obtain data from multiple sources and examine an appropriately sized catchment area to optimize accuracy of incidence calculations. Examination of the latter, however, is limited by the lack of published population-based studies for large parts of Africa, Latin America and Asia. Some migrant studies have suggested the lower incidence in these populations may be real. For example, in the United States, the prevalence of ALS among African-American was half that seen among those of European ancestry. In contrast, a UK urban population-based study did not find the same difference, suggesting that the difference observed in the US population may reflect differences in socioeconomic status and access to healthcare across different ethnicities. Still, lower ALS mortality rates have been seen in populations of mixed ancestry, implying that genetic admixture may be protective as regards ALS risk. It is estimated that the number of prevalent ALS cases will increase by nearly 70% in the next 25 years with developing regions in Asia, Africa and Latin America expected to see the greatest increase in disease prevalence owing to the rapidly changing demographic profiles (increasing population and life expectancy).

This provides an even greater onus for studying ALS epidemiology in these populations to identify the public health needs and ensure equitable resource allocation.

1.11.1. Gender, age and survival in ALS

Male sex and advancing age are the only established risk factors in ALS. Males are more likely to develop ALS compared to females with a reported male: female ratio with respect to disease incidence of approximately 1.3:1, the disparity explained by the increased incidence of spinal onset ALS among males. ALS incidence increases with age, peaking in those in their late sixties and early seventies. The incidence of ALS decreases rapidly after eighty years of age. While this could reflect difficulties with case ascertainment in the elderly due to difficulties excluding mimic disorders and the increased risk of death before formal diagnosis in this population, this pattern could also suggest that ALS is a disease that occurs within a susceptible group in the population rather than being primarily a disease of aging.

ALS disease phenotypes shows sex-specific differentiation. While spinal onset disease is more common than bulbar onset disease in younger females, this pattern shifts with increasing age such that female patients over eighty years of age are more likely to present with bulbar onset disease. In contrast, male patients are more likely to present with spinal onset disease, regardless of age. Other studies have reported on an increased risk of respiratory onset disease and flail arm variants of ALS in men and executive dysfunction among female ALS patients. The reasons behind this apparent
age-related selective vulnerability regarding the focality of onset remains as of yet incompletely understood and may reflect site-specific factors\textsuperscript{237}.

Increasing age at symptom onset is a strong negative prognostic factor in ALS\textsuperscript{219}. One study found that patients with symptom onset in their late fifties and early sixties survived approximately 2 to 3 times longer than those whose symptoms developed after the age of sixty-five\textsuperscript{238}. In very late-onset ALS (aged 80 years or older at time of symptom onset), disease survival was found to be significantly shorter than for younger onset patients\textsuperscript{239}. The interplay between gender, site of onset and age of symptom onset and how they relate to disease survival is complex. Both bulbar onset disease and female gender are reported to be poor prognostic factors in ALS, although there is conflicting data on the latter\textsuperscript{219}. Further complicating the issue, is the finding that C9orf72 repeat expansion carriers develop ALS at a later age in female and bulbar onset cases\textsuperscript{240}. In a multicentre European study, which assessed data on 11475 patients with ALS with an aim to develop a multivariate predictive model for ALS survival, age of symptom onset, bulbar onset disease and carrying the C9orf72 repeat expansion were found to independent significant survival predictor variables while female gender was not found to be a significant predictor independently\textsuperscript{221}. While genetic factors may influence age onset and survival, other biological reasons such as the greater loss of neuronal reserve with increasing aging\textsuperscript{241} may also contribute to poorer survival in elderly patients.

1.12. The ‘Familial’/’Sporadic’ ALS dichotomy

ALS may be categorized into ‘familial’ and ‘sporadic’ forms. Following the publication of a case series in the 1950s\textsuperscript{242, 243}, the incidence of familial ALS is frequently reported to be 10%. However, more recent studies have shown that this figures varies markedly\textsuperscript{244}, in large part due to inconsistencies between studies in their design and how familial disease is defined. Most ALS researchers agree that the presence of at least first-degree relative with ALS constitutes familial disease\textsuperscript{245}, yet this consensus dissipates with the degree of relatedness of affected relatives (e.g., second- degree and more distant relatives). Likewise, there is a lack of consensus over whether the presence of relatives with related disorders (e.g., FTD or schizophrenia) should be included in what constitutes familial ALS, with clinicians with more experience and a specialist interest in ALS more likely to concede the clinical relevance\textsuperscript{245}.

In contrast, the situation may arise where an ALS patient with no apparent family history of ALS, may carry a known causative ALS mutation. The apparent sporadic nature of this case may be attributable to a number of factors including small family size, the premature death or misdiagnoses of relatives, lack of available information on relatives,
denial or low gene penetrance\textsuperscript{246}. Some authors argue that for these reasons the dichotomy of familial and sporadic ALS has outlasted its utility\textsuperscript{247}. In lieu, it is proposed that ALS is categorized by whether it has a genetically identifiable cause or not (genetic versus non-genetic ALS). Undoubtedly, this is an important clinical distinction, particularly in light of emerging therapies targeting specific genes.

Nonetheless, this dichotomy too has its limitations. Not all ALS causative genes have been identified, meaning some cases of genetic ALS will be miscategorized as non-genetic. Furthermore, this distinction assumes a simple monogenic model for ALS and does not allow for the complexity of oligogenic models and the roles played by other genetic and environmental modifiers on gene expressivity. While less than ideal, the concept of familial ALS will likely continue to be used for guidance in genetic testing decisions, at least for the near future\textsuperscript{245}. In a research context, the familial versus sporadic distinction serves at least three purposes:

1. To identify families with multiple affected individuals in whom no gene has yet been identified. These families may be suitable for linkage studies.

2. To characterise the expanded phenotype associated with ALS and particular genes.

3. To allow for the fair comparison of the composition of different populations in genetic epidemiology studies.

Considering all of this, consensus diagnostic criteria for familial ALS have been proposed with varying levels of stringency\textsuperscript{246}. The most stringent definition is that of “Definite familial ALS” which describes case where the patient with ALS has two or more first or second-degree relatives with ALS OR at least one relative with ALS and gene-positive co-segregation. The least stringent criteria “Possible familial ALS” encompasses patients with ALS who have an ALS causative genetic mutation but no family history of the disease. It also encompasses patients with ALS who have a relative with confirmed FTD or a distant relative (third-degree or more) with ALS.

### 1.13. Genetic architecture of ALS and “Clan Genomics”

Causative genetic mutations have been identified in close to 70% of familial ALS cases\textsuperscript{248}, predominantly in C9orf72, SOD1, TARDBP and FUS genes\textsuperscript{249}. Mutations in these major ALS genes have also been identified in over 10% of apparently sporadic ALS cases\textsuperscript{248}. A modelling study by Al-Chalabi et al (2011)\textsuperscript{250} has shown such apparent sporadic patterns may emerge in Mendelian gene carriers as a consequence of incomplete gene and family size with the rate of apparently sporadic disease expected
to increase as population demographics change. This is concordant with work by Van Rheenen et al (2016)\textsuperscript{251} who identified that multiple rare variants play a key role in the development of sporadic ALS. As such, the genetic architecture of ALS can be considered markedly different from other complex diseases such as schizophrenia which result from the additive effects of numerous small effect common variants\textsuperscript{252}.

Such common variants still have a small role to play in ALS, with polygenic risk accounting for an estimated 8\% of disease heritability\textsuperscript{251}. However, by contrasting this estimate with those obtained from twin-based studies, McLaughlin et al (2015)\textsuperscript{253} highlights how the missing heritability in ALS is best explained by the existence of numerous, rare and possibly frequent de novo mutations, and advocates for detailed genome sequencing studies of ALS kindreds to expedite the discovery of inherited variants arising individual pedigrees. In each individual, their genome can be considered to consist of common variants specific to their population, inherited rare variants arising in their recent family lineage and de novo mutations, with the latter two scenarios having a greater impact on an individual’s disease susceptibility\textsuperscript{254}. Population variants may be targetable as pharmacogenomics traits. This concept of “clan genomics” where rare variants arising from recent family lineage play a causative role in disease is displayed in Figure 1-4 below.
1.14. Genotype-phenotype confounders

The Mendelian framework proposes that monogenic diseases develop as a result of one dominant or two recessive allelic variants in one gene. Yet the development of ALS phenotypes is rarely as specific and predictable as this model would purport. Numerous genetic mutations are associated with near identical clinical presentation (genetic heterogeneity) with few distinguishing correlations identified between specific variants with regard to site of onset, disease progression and survival. Furthermore, bar differences in mean age of disease onset, for most cases there are no clear distinguishing features between gene carriers and apparently sporadic ALS. By contrast, individuals carrying one pathogenic ALS gene mutation may never develop the disease (incomplete penetrance), may manifest the disease phenotype to a greater or lesser severity (variable expressivity) or develop a separate or overlapping clinical syndrome (genetic pleiotropy). These terms provide phenotypic level nomenclature but do not explain the underlying molecular mechanism involved\textsuperscript{255}. Oligogenic inheritance or genetic interaction/modification are often used interchangeably to describe the
modulation of the phenotypic outcome of one gene/allele by a second gene/locus. The distinction between these terms lies in whether or not the primary locus is both necessary and sufficient to cause the disease? If yes, the role of second gene/locus plays is purely that of a modifier of the severity of phenotype. In contrast, in cases of oligogenic inheritance the presence of the second gene/locus is required for the disease to occur\textsuperscript{256}. I will discuss briefly the oligogenic model of ALS before expanding on other putative genetic modifiers in C9orf72 associated ALS.

1.14.1.1. The oligogenic model of ALS

An oligogenic model of ALS was first proposed by van Blitterswijk et al (2012)\textsuperscript{257} following completion of a study examining complex inheritance models in 97 familial ALS families as well as sporadic ALS patients and control subjects. Participants were screened for mutations in TARDBP, FUS/TLS, SOD\textsubscript{1}, ANG and C9orf72 repeat expansion. In 5/97 familial ALS families, multiple mutations in ALS-associated genes were observed, a frequency higher than expected by chance alone. FUS/TLS and TARDBP mutations occurred in combination with ANG mutations and C9orf72 repeat expansions with TARDBP, SOD\textsubscript{1} and FUS/TLS mutations. The strongest evidence in support of the hypothesis was found in 15 patients carrying a p.N352S mutation in TARDBP, 50\% of whom had an additional mutation\textsuperscript{257}.

This work has been replicated by other authors. A UK study using next generation sequencing data from 1126 ALS patient samples and 613 controls, found 11 patients with more than one mutation, a significantly higher probability than expected by chance alone as determined by binomial analysis of the mutation rates in cases and controls\textsuperscript{258}. Among Italian ALS-FTD pedigrees carrying the C9orf72 repeat expansion, 4/11 pedigrees were found to carry additional variants in ALS or dementia-related genes, with double mutations carriers displaying a tendency towards a younger age of onset and the development of Parkinsonism\textsuperscript{259}. This tendency towards a younger age of onset was also observed in multiple mutation carriers (n=15, 3.8\%) identified in a US study using targeted pooled-sample sequencing of 17 ALS genes in 391 ALS patients\textsuperscript{260}. Furthermore, one large Australian study of whole genome sequence data from 616 sporadic ALS patients, observed that 6.8\% of cases harboured two or more known ALS-linked mutations. Sporadic ALS cases with a younger age of onset were found to be more likely to carry multiple ALS associated variants\textsuperscript{261}. A separate Italian study of 83 familial and sporadic ALS patients, using targeted next generation sequencing (NGS) data, enriched in ALS-associated genes, identified multiple gene variants in 17\% of
patients. Higher rare variant burden was associated with reduced survival, independent of other known negative prognostic factors\textsuperscript{262}.

However, a word of caution is advised by Keogh et al (2018)\textsuperscript{263} with respect to the oligogenic model in ALS. The authors studied 980 neuropathologically characterised human brains, including those of 244 people with ALS-FTD and 362 age-matched controls, using targeted exome-sequencing of 28 ALS-FTD associated genes among other panels. The majority of ALS-FTD patients with two or more gene variants (n=19, 7.8\%) carried one highly penetrant allele or known risk factor in combination with another rare and likely benign allele, with the frequency of the oligogenic variation in different cohorts linked to the size of the gene panel and MAF threshold used. The authors caution against the potential introduction of systematic bias where affected individuals, and in particular those with familial disease, are more likely to harbour a known genetic risk. As such, when one considers the background variant carrier rate, they are subsequently more likely to be classified as oligogenic than healthy controls. Exclusion of those with known pathogenic variant, removed the apparent association between oligogenic carriers and younger age of disease onset.

This cautionary tale does not preclude the hypothesis the oligogenic model may have an important role to play in ALS disease risk and phenotypic expression, but points to the difficulties inherent in determining how these apparent “additional” variants may impact disease risk and phenotypic modification. Attempts to answer these questions must require comprehensive sequencing of all known genes in large cohorts of patients with a rare disease, also taking into account the background carrier variant rate. Yet determining the extent to which oligogenic interactions drive disease risk and phenotypic expression or not has important clinical and research implications. For example, patients with known ALS gene mutations may often be excluded from studies designed to identify new ALS associated genes and it is argued that this should not be the case\textsuperscript{257}.

1.14.1.2. \textit{C9orf72 phenotypic modifiers}

The \textit{C9orf72} repeat expansion is markedly pleiotropic mutation. While most mutation carriers will develop ALS and/or FTD, numerous other clinical syndromes have been linked to the mutation, including schizophrenia and other psychiatric disorders, Parkinsonism, Alzheimer’s disease, Huntington’s disease phenocopy, progressive supranuclear palsy (PSP), corticobasal degeneration, sporadic spinocerebellar ataxia, olivopontocerebellar degeneration, seizures and intellectual disability\textsuperscript{264-267}. Furthermore, with respect to ALS, the repeat expansion is associated with marked
variability in clinical presentation. The C9orf72 repeat expansion is linked to the full spectrum of motor neuron diseases, from isolated upper motor neuron involvement (PLS) to lower motor neuron disorders (PMA)\(^\text{220}\). While at a group level, the repeat expansion is consistently associated a younger mean age of onset, individual carriers may develop ALS from their early 20s up until their 90's\(^\text{240, 265}\). Equally, a meta-analysis of over 1000 C9orf72 repeat carriers, reported differences in survival from onset ranging from months to decades for carriers with ALS (n=455, median 2.83 years, range 0.17 – 14), ALS-FTD (n=198, median 3 years, range 0.33 – 19) and FTD (n=296, median 9 years, range 1 – 31)\(^\text{268}\). Older age at onset was associated with worse prognosis for carriers in all cohorts, with bulbar onset disease associated with shorter survival in C9orf72 positive ALS. This remarkable phenotypic heterogeneity points to the existence of the genetic modifiers for this repeat expansion. Indeed, some putative gene-gene interactions have been identified in C9orf72 carriers. Intermediate ATXN2 repeat lengths have been associated with a higher risk of developing ALS among C9orf72 carriers\(^\text{269}\). Repeat expansion carriers carrying additional mutations in ubiquitin-associated protein 1 (UBAP1), prion protein (PRNP) and metallothionein 1 E (MT-Ie) showed an earlier age of disease onset for ALS and FTD, with mutations in GRN associated with shorter disease survival\(^\text{270}\). The same authors also reported that homozygosity for minor alleles of transmembrane protein 106 B (TMEM106B) variants may protect C9orf72 carriers from developing FTD but not ALS\(^\text{271}\).

Furthermore, as with other repeat expansion disorders, the impact of repeat expansion length on phenotypic manifestations has been of much interest in C9orf72 associated disease. In people with ALS and/or FTD, repeat expansion lengths of several hundreds to thousands can be found\(^\text{140, 266}\). While occasionally repeat expansion lengths in this range can be detected in a healthy population\(^\text{266}\), in most healthy people, a repeat expansion length of 1-2 units is most commonly observed, followed by 5-repeat, 6-repeat, 7-repeat or 8-repeat unit\(^\text{272}\) with 90% of healthy people of European ancestry carrying between 2-10 repeats\(^\text{273}\). While the exact upper limit for repeat expansion length is unknown due to limitations of testing, there remains also uncertainty about the lower limit for pathogenicity. 30 or more repeat units has generally been accepted as the cut-off point between pathogenic and neutral expansions, since the original descriptions. This is primarily due to technical limitations of the repeat-primed PCR technique\(^\text{141, 272}\). More recently the argument has been made, for consideration of lowering the threshold for pathogenic expansions to 24 or more repeats\(^\text{274}\).
Intermediate length repeat expansions are variably defined and of less certain importance. In several studies, an intermediate expansion length definition of between 20-29 repeats has been used\textsuperscript{275}. 4 FTD families were found to carry repeat expansions in this range with co-segregation of the expansion with disease, which was often preceded by psychiatric symptoms\textsuperscript{276}. Similarly, Byrne et al (2014)\textsuperscript{275} report on 4 ALS with intermediate expansions (range 20-22 repeats), all of which had a family history of FTD or unspecified dementia or psychiatric disorders. These patients also exhibited a younger age of onset than ALS patients who carried less than 20 repeats. FTD-like dementia and other neuropsychiatric symptoms were also observed in a small cohort of patients with atypical parkinsonism associated with intermediate expansions (range 20-29 repeats)\textsuperscript{277}. However, in an Irish psychosis case-control study (n=2477), no overall evidence of association between schizophrenia and repeat expansion length was detected with intermediate repeat expansions recorded in just two schizophrenia cases (range 27-28 repeats) and five controls (range 23-26 repeats)\textsuperscript{278}. Overall, at what value the threshold for pathogenicity for different disease phenotypes should be set and the exact relationship between intermediate expansion length and neuropsychiatric symptoms remains unclear\textsuperscript{272}, with efforts to answer these questions hampered by its very low frequency of occurrence\textsuperscript{274}.

1.15. Environmental risk factors for ALS

Attempts have been made to determine the impact of numerous environmental stressors on ALS risk and phenotypic manifestations. Such studies have often been limited by inherent difficulties with identifying and quantifying such exposures over a lifetime. Few studies yielding high-quality evidence are available as most studies are small, retrospective, not population-based and do not account for potential gene-environment interactions\textsuperscript{139}. The only established risk factors in ALS are male sex and advancing age\textsuperscript{233}. Table 1-8 summarizes the results of a meta-analysis\textsuperscript{279} examining the evidence supporting some other putative environmental risk factors. Occupation and lifestyle factors are discussed separately as they relate to multiple strong correlated risk exposures.

Cigarette smoking has long been considered an established risk factor for ALS\textsuperscript{280} and is supported by data from a large European population-based prospective study showing that cigarette smoking increases ALS risk, irrespective of sex\textsuperscript{281}. Yet, a recent meta-analysis of 20 case-control studies did not find this association, although not all studies included in this analysis were population-based\textsuperscript{279}. The mid-20\textsuperscript{th} century clusters of ALS/PDC in Guam and Kii peninsula incited a slew of studies on environmental exposures in these regions. One popular hypothesis links dietary beta-N-methyl-ano-
L-alanine (BMAA) to the endemic foci in Guam, supported by the observation in small studies of increased BMAA concentrations in brains of people with ALS compared with controls, although this work requires replication. As cyanobacteria produce a variety of toxins, including BMAA, this could partially explain the apparent spatial clustering of ALS near algal bloom outbreaks in France, Japan, New Hampshire and Wisconsin. Still, recent work identifying an increased burden of genes linked to ALS, parkinsonism, dementia and other neurodegenerative disorders among the Guamanian Chamorros, underlines the importance accounting for potential gene-environment interactions, as such environmental exposures may only be relevant in a genetically predisposed population.

Viral infections, including endogenous retroviruses have also been proposed as putative risk factors for ALS, supported in part by the observation of increased reverse transcriptase activity in sera from ALS patients and their relatives, but not spouses or healthy controls. This work suggests the possibility that an inherited endogenous retrovirus may predispose to the development of ALS in a synergistic manner similar to that observed in other human diseases (e.g. Burkitt's lymphoma). This has offered the opportunity for novel therapeutic option targeting a human endogenous retrovirus (HERV), HERV-K (HML-2), with an anti-retroviral therapy Triumeq (abacavir, lamivudine and dolutegravir) which has demonstrated some early success in slowing disease progression in a phase II study, paving the way for a larger international phase 3 trial of this compound for use in ALS.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Papers No</th>
<th>Patient</th>
<th>Controls</th>
<th>Significance</th>
<th>OR (95% CI)</th>
<th>I² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metals</td>
<td>10</td>
<td>1459</td>
<td>1887</td>
<td>&lt;0.00001</td>
<td>1.71 (1.38, 2.11)</td>
<td>0</td>
</tr>
<tr>
<td>Pesticides</td>
<td>10</td>
<td>2001</td>
<td>99405</td>
<td>0.0007</td>
<td>1.48 (1.18, 1.86)</td>
<td>40</td>
</tr>
<tr>
<td>Organic Solvents</td>
<td>7</td>
<td>701</td>
<td>1286</td>
<td>0.007</td>
<td>1.43 (1.10, 1.86)</td>
<td>16</td>
</tr>
<tr>
<td>Previous trauma</td>
<td>17</td>
<td>2955</td>
<td>3763</td>
<td>&lt;0.00001</td>
<td>1.73 (1.43, 2.09)</td>
<td>53</td>
</tr>
<tr>
<td>Electric shock</td>
<td>6</td>
<td>961</td>
<td>1154</td>
<td>&lt;0.00001</td>
<td>3.27 (1.87, 5.73)</td>
<td>0</td>
</tr>
</tbody>
</table>

1.15.1. Occupation

Small studies and case series have suggested an increased risk of developing ALS associated with numerous varying professions and industries. Industries such
agriculture, fishing, construction work and the military share some common occupational exposures, considered putative risk factors for ALS, include exposure to heavy metals, pesticides, chemicals, dust irritation, air pollution, extremely low-frequency electromagnetic fields and requirements for intense physical activity. A meta-analysis of 22 studies exploring the relationship between ALS risk and rural environmental exposures found an increased ALS risk associated both with agricultural work and pesticide exposure. The risk of developing ALS was also found to be increased by approximately 20% for those working in forestry or construction, possibly linked to exposure to toxins such as diesel exhaust fumes and lead. Research on military personnel with regard to ALS risk has drawn much interest as people in this field of work are exposed to numerous potential injurious agents including physical and psychological trauma and exposure to various toxins. A meta-analysis of 8 case-control studies and 3 cohort studies confirmed an increased risk of ALS associated with military employees (pooled OR 1.29 95% CI: 1.08–1.54). Numerous putative environmental risk factors have been suggested to explain the increased risk of ALS observed in professional footballers, including repetitive head trauma and chronic exposure to pesticides (on football pitches) or illegal performance enhancing substances.

1.15.2. **Lifestyle**

Conversely, the higher prevalence of ALS seen among some athletes, may be viewed as a natural continuation of reported pre-morbid lifestyle factors associated with ALS risk. Higher levels of physical activity, lower pre-morbid BMI and fewer cardiovascular risk factors have all been associated with increased ALS risk, with the question outstanding being whether these factors independently impact risk or in themselves reflect a shared genetic predisposition. Retrospective studies have reported on higher levels of leisure time physical activity during adulthood among ALS patients compared with controls, with a prospective Swedish register study demonstrating an association between physical fitness at an early age and ALS risk. While patients with ALS are usually lean with a normal or lower BMI compared with the healthy population, examination of impact of pre-morbid BMI on ALS risk can be complicated by the fact that patients with ALS may experience weight loss as part of the disease process. A Norwegian population-based study by Nakken et al (2019) demonstrated an inverse relationship between increasing pre-morbid BMI and ALS risk approximately 30 years later, with the risk of developing ALS decreasing by between 12-21% with every 5-unit increase in pre-morbid BMI. This work was replicated in a meta-analysis by Zeng et al (2019) which again showed that ALS risk reduced by 3.0% (95%
CI 2.1-4.5%) for every unit increase in BMI. The “fitness lifestyle hypothesis” also links with an inverse relationship between cardiovascular and ALS risk, which is supported by epidemiological studies chronicling less co-morbid and antecedent cardiovascular disease and cardiovascular risk factors (type 2 diabetes mellitus, hypertension, hyperlipidaemia) among ALS patient populations\textsuperscript{300-303}.

Yet, in counter to the argument that the fitness lifestyle profile directly increases ALS risk, is conflicting evidence regarding the dose-response relationship between physical activity and ALS risk\textsuperscript{291, 294} and the lack of evidence supporting altered disease course in those with ALS with other cardiovascular risk factors\textsuperscript{300, 305}. This suggests that the fitness phenotype may result instead from a common genetic profile conferring increased ALS susceptibility\textsuperscript{294, 306}. Rather than cardiovascular risk factors protecting against ALS, the converse may be true with the pathophysiological underpinnings of ALS protecting against cardiovascular risk\textsuperscript{301}. This “hypervigilant hypothesis” purports that the existence of aggressive regulation of homeostasis balance with resultant feedback gains would result in initial protection against a variety of conditions but ultimately result in damage to motor neurons which are susceptible to regulatory delay\textsuperscript{301}. Further research is required to assess the validity of this hypothesis.

1.16. Multistep model of ALS

The clinical onset of ALS typically occurs later in life and is marked by rapid deterioration from a seemingly asymptomatic baseline, even in people who have inherited a known ALS-causative gene mutation. Furthermore, many people who carry such mutations may never develop the disease or may develop other neurodegenerative disorders. Tying these clinical characteristics together with the genetic and environmental risk factors described above is the multistep step model of ALS proposed by Al-Chalabi et al (2014)\textsuperscript{307}. This model, developed from a prototype applied in cancer epidemiology\textsuperscript{308} suggests that development of ALS is a six-step process. While the original age-specific incidence data was derived from five separate population-based registers in Ireland, England, Scotland, Netherlands and Italy, this work has since been replicated including Australia, South Korea and Japan, with a slightly higher slope estimate (extra half step) in the latter population\textsuperscript{309}.

Chiò et (2018)\textsuperscript{310}, using an identical methodological approach applied to Italian and Irish ALS register data, identified that the number of steps required to develop ALS was reduced in those ALS-causative genetic mutations. The model suggests that SOD1 mutation carriers require two steps, C9orf72 repeat expansion carriers three steps and
TARDBP carriers four steps, to develop ALS. Overall familial cases were estimated to necessitate a four-step process, with those negative for known ALS-causative genes requiring an additional step (five-step process). In contrast, the full six-step process was seen in “sporadic” ALS patients without known ALS-causative genes. The model fits with the observation of earlier mean ages of onset in familial ALS cases and work showing a significantly younger age of onset for among sporadic ALS gene carriers compared to sporadic ALS patients without known gene mutations.

A recent expansion of the model by Garton et al (2021) combination Danish population-based register data with a metanalysis of data from previous studies, showed the multistep model was a half-a-step fewer for men. The authors found no difference in the number of steps required for those with a prior history of psychiatric disorders. However, caution should be advised with the interpretation of this study that psychiatric disease is not a risk factor for ALS as only those receiving in-patient or out-patient treatment for their psychiatric illness were considered and all psychiatric disorders were grouped together. As such, the impact of disorders with the strongest genetic linkage to ALS (e.g., schizophrenia/psychotic disorders) may be masked by those with weaker association but of greater prevalence in the general population (e.g., anxiety) and the impact of more subtle pre-morbid personality traits would be missed altogether.

1.17. Conclusion

In this chapter, I have introduced ALS, a progressive neurodegenerative disease, which is heterogenous in its clinical presentations, but relatively uniform in its dismal prognosis. ALS is considered to exist on a spectrum with FTD, with strong genetic, pathological, anatomical and clinical evidence supporting an overlap between both disorders. More recent epidemiological evidence would suggest that this spectrum should be widened to encompass other neuropsychiatric disorders, but further work is needed to explore the shared genetic underpinnings. In this chapter, I have also introduced the clan genomics model as a way to consider genetic risk inherited from distal ancestors (common variants) and more recent ancestors (rare variants). In the next chapter, I will use this model to consider how both population-specific and familial genetic risk may contribute to variance in the phenotypic presentation of ALS.

2.1. Introduction

The common question asked with respect to ALS aetiology is the relative contributions of genetic versus environmental risk exposures. Answering this question can help us in identifying those at greatest risk, understand the underlying disease processes and determine how to best to intervene to prevent or ameliorate the risk. Disease heritability is a term used to describe the extent to which variation in disease risk is attributable to genetic factors. However, it is important to remember that while this term is often used interchangeably to mean 'genetic contribution', heritability refers specifically to population rather than individual-level risk. Instead, the clan genomics model discussed in Chapter 1. may be used to describe an individual's genomic disease risk profile which includes both population-specific common variants inherited from distal ancestors and rare but potentially larger effect variants inherited from more recent ancestors, with the latter believed to have greater impact on an individual's disease susceptibility.

In this regard, ALS heritability estimates, derived used different methodological approaches, have been useful in informing us of the relative importance of population versus familial risk in ALS. Work by Keller et al (2014) and Fogh et al (2014) on ALS heritability as captured by common SNPS show that population genetic sub-structure plays an important role in ALS risk, with ongoing and larger GWAS studies warranted to explore this avenue. By contrast, the apparent “missing heritability” between these estimates and the much higher estimates derived from pedigree studies, may be best explained by the occurrence of numerous, rare mutations arising within families, with such variants now believed to account for a large proportion of ALS risk even in sporadic ALS cases. Following from this, it is clear that any thorough examination of genetic risk in ALS should account for both population and familial variants. Detailed genotype-phenotype studies in kindreds with familial clustering of ALS likely to be particularly informative. In this chapter, I will discuss (1) what is known about the impact of population genetic on ALS risk and phenotype, (2) how genetic burden manifests within ALS kindreds and (3) how novel endophenotypic approaches could be adopted in ALS to aid in the discovery of new ALS associated genes.
2.2. Population Genetics and ALS Risk

2.2.1. Background

The vast majority of ALS epidemiological and genetic studies to date have been conducted in populations of European ancestry. The genetic sub-structure of European populations is likely determined for the most part by the spread of early farmers from Levant, with some input of genes from other sources including North Africa and Asia\textsuperscript{320}. Over time, geographic and linguistic barriers gave rise to marked local divergence stimulating regional founder effects which contribute in a small but important way to modern genetic variation\textsuperscript{320}. Recent studies of the European genetic substructure have shown that populations of Northern European ancestry can be reliably differentiated from those of Southern European ancestry\textsuperscript{321, 322}, with an east to west gradient observable among Northern Europeans\textsuperscript{321}. On a larger scale, this historical population genetic diversification in geographically isolated regions, also gave rise to distinct relatively genetically homogenous continental population groups (e.g., European, African, Asian). Subsequently, major historical events including the conquest and colonization of the Americas and the trans-Atlantic slave trade led to high levels of genetic admixture in some Latin American countries. These populations may comprise various groups of differing Native American, European and African ancestral origins\textsuperscript{323}. For example, in Cuba, the population can be genetically geographically stratified by ancestral origin with Eastern provinces showing higher African and Native American contributions than Western regions\textsuperscript{324}. These populations offer the unique opportunity to examine how differing population genetic backgrounds impacts on disease risk among those living in a shared environment and how this risk may be impacted by genetic admixture.

In contrast, the Irish population may be considered more genetically homogenous than other European populations. Runs of homozygosity are found to be longer and more frequent in Ireland than in Britain, and interestingly were also longer and more frequent in Irish ALS patients compared to population-matched controls, supporting the hypothesis that ALS risk may be higher in less genetically diverse populations\textsuperscript{325}. While work by Byrne et al (2018)\textsuperscript{326} has demonstrated that even within a genetically homogenous population, regional genetic substructures may exist arising from historically low ancestral mobility within Ireland. Nonetheless, the small size and island status of Ireland, the historically low immigration rates and larger family sizes and the existence of a long-standing population-based ALS register makes Ireland an ideal population in which to examine genetic risk factors associated with ALS.
2.2.2. Geographic variance in known ALS genes

The importance of population-specific assessments is reflected in the variation in the incidence of familial forms of ALS across differing geographical regions. A meta-analysis on familial ALS incidence observed familial ALS rates of between 5 – 20% across European populations244 which may stem from heterogeneity of the population genetic sub-structure327, but could also reflect differences in study design and inconsistencies in the definition of familial ALS adopted across various studies244. With respect to known ALS-causative genes, significant variation across different continental populations is observed although it is notable that studies in non-European populations are often small and derived from clinic-based populations. The most important ALS genes consistently identified across various European populations are the pathogenic C9orf72 repeat expansion and mutations in SOD1, TARDBP and FUS. In European populations, mutations in these four genes account for 55.5% of familial ALS cases (C9orf72 [33.7%], SOD1 [14.8%], TARDBP [4.2%], FUS [2.8%]) and 7.4% of sporadic ALS cases (C9orf72 [5.1%], SOD1 [1.2%], TARDBP [0.8%], FUS [0.3%])249. In contrast, among Asian populations these mutations are less common accounting for only 40.2% of familial ALS cases (SOD1 [30.0%], FUS [6.4%], C9orf72 [2.3%], TARDBP [1.5%]) and 2.9% of sporadic cases (SOD1 [1.5%], FUS [0.9%], C9orf72 [0.3%], TARDBP [0.2%])249. The C9orf72 repeat expansion was found to account for 9% of ALS cases of Cape mixed-African ancestry (familial status not differentiated)328 and approximately 3% of sporadic ALS cases in Brazil and Argentina329, 330.

Within Europe, where most genetic epidemiological studies have been performed, marked variation in the incidence of the C9orf72 repeat expansion, SOD1, TARDBP and FUS has also been observed across different populations249. The C9orf72 repeat expansion was most frequently detected in Finnish and Greek populations (Finland 46.4% FALS, 21.1% SALS; Greece 50% FALS, 8.1% SALS). The frequency of SOD1 was highest in Russian and Scottish populations (Russia 50% FALS, 10% SALS; Scotland 50% FALS, 7% SALS). TARDBP was detected most frequently in Sardinian, followed by Italian populations (Sardinia 35% FALS, 22.5% SALS; Italy 12.5% FALS, 7.3% SALS). Finally, the frequency of FUS mutations was highest in Italy (7.5% FALS, 1% SALS). The relatively high incidence of certain genetic mutations in different regions is likely driven by the presence of population isolates. The C9orf72 repeat expansion is widely linked to a Finnish haplotype331 which is believed to have arisen in Northern Europe approximately 1500 years ago. The same haplotype has since been identified in association with the repeat expansion among ALS patients more than 17 regions
worldwide suggesting that this mutation occurred once in human history and through human migratory practices has become disseminated throughout various populations. In a similar manner, a large proportion of Sardinian ALS cases are attributable to a single founder mutation of the TARDBP gene. A single missense mutation accounts for a third of all ALS cases on the genetically isolated island, all of whom share a large risk haplotype across the TARDBP locus, highlighting the potential insights which may be offered by performing genetic studies in such isolated populations. In Scotland, the relatively high incidence of SOD1 ALS is accounted for by the I114T variant, which is believed to have arisen from a local founder mutation approximately 250 years previous. In contrast, the SOD1 AV4 variant which is the most frequent SOD1 mutation in the United States, but rare among European populations, is likely to have originated in the Native American population approximately 12000 years ago.

In the Irish population, the C9orf72 repeat expansion is the only notable genetically identifiable cause of ALS accounting for 41% of familial ALS cases and 5% of those with sporadic ALS. All C9orf72 carriers have the same common haplotype found in other European populations. Furthermore, an Irish population-based high-throughput sequencing study of ALS gene variants also identified 4 ALS patients carrying other known ALS variants (TARDBP: c.859G>A(p.[G287S]) [n=2]; FUS: c.1574C>T(p.[P525L]) [n=2]). The 4 variants were detected in patients with no family history of ALS (0.45% sporadic ALS), with all mutation carriers remaining cognitively intact during the course of the disease. Those carrying the FUS mutations had a younger age of disease onset and a rapidly progressive disease course, consistent with previous reports. Compared to Italian ALS patients, Irish people with ALS are more likely to carry the pathogenic C9orf72 repeat expansion and less likely to harbour pathogenic SOD1 and TARDBP mutations. No detectable excess in apparent oligogenic inheritance patterns was observed among Irish ALS patients, with just 1.6% of patients carrying two or more known or potential ALS variants. Notably both TARDBP mutation carriers carried a second putative ALS mutation, which aligns with evidence from a separate Dutch study which found the strongest-evidence in support of the oligogenic model of ALS among TARDBP mutation carriers.

2.2.3. Geographic variation in ALS phenotype

As discussed in Chapter 1, the incidence of ALS varies across different geographical regions. While the incidence of ALS in populations of European is relatively homogenous, strong heterogeneity in incidence rates is observable between European and Asian populations. Reports of an apparent north-to-south gradient or even east-
to-west ALS risk gradient have been recounted\textsuperscript{224}. Within Europe there is an apparent trend for increasing ALS incidence rates with increasing latitude\textsuperscript{338, 339}, with the highest risk of ALS in Scandinavia, possibly reflecting spread of high risk ALS variants\textsuperscript{227}. Similarly, in the US, a large mortality study\textsuperscript{340} reported on an increasing risk of death due to ALS with increasing northwest gradient which may arguably reflect on the increasingly uniform population genetic background. In contrast, no high-risk regions were identified in a spatial cluster analysis of the more homogenous Irish population\textsuperscript{341}.

Geographic variance in some ALS clinical features has also been reported. The mean age of onset is lower in Asian populations compared with European, with longer median survival durations in the former\textsuperscript{337}. Shorter survival durations were also in northern compared with southern Europeans, reflecting the higher proportion of bulbar onset disease in the former. In Asia, bulbar onset disease was even less common. While it is interesting to speculate as to factors underlying geographical variation in ALS incidence and phenotype, distinguishing what is attributable to underlying genetic profile, environmental exposures, and demographic, socioeconomic and healthcare differences is a momentous task. The current literature suggests the greatest difference in ALS risk and phenotypic manifestations can be observed between populations of different continental backgrounds, with some notable differences attributable to local founder effects. Epidemiological studies of admixed populations, including those in Latin America, therefore offer a great opportunity to examine populations of different ethnic background living in the same territory allowing researchers to decipher the relative impact of both distinct genetic and shared environmental risk factors.

\textbf{2.2.4. Summary}

Significant geographical variation is seen in both the incidence and prevalence of ALS and the incidence of known ALS gene mutations. While it is tempting to correlate the former with the latter, there are many other factors at play which could account for some of the heterogeneity observed, including differences in study design, case ascertainment, cohort choice etc. Furthermore, in many parts of the world, there is simply insufficient data to comment on the population ALS risk. Nonetheless, the bulk of the evidence suggests the population genetic background probably contributes to some degree to the diversity of ALS risk and presentations. Studies in admixed population have utility in assessing those of different ethnic backgrounds who share similar environmental exposures.
2.3. Genetic Burden within ALS kindreds

2.3.1. Risk of ALS among Relatives

Heritability studies examining twins of probands have shown they are at increased risk of developing ALS\textsuperscript{342, 343}. Similarly parents of probands have been observed to be at increased risk with Wingo et al (2011)\textsuperscript{344} reporting 1.1% of parents of ALS probands developed ALS in their clinic-based pedigree study. Studies estimating ALS heritability have in themselves been besieged by issues of insufficient power and methodological limitations. In rare diseases like ALS, it can be particularly difficult to recruit sufficient numbers of monozygotic and dizygotic twins to study disparities in phenotypic concordance\textsuperscript{342, 343}. The pedigree study by Wingo et al (2011)\textsuperscript{344} provides the best estimate of ALS heritability as being between 40 – 60%. Yet, even in this large study, estimates were derived using indirect reported prevalence data rather than direct ALS incidence estimates for the population studied.

Two studies report an 8 to 10-fold increased lifetime risk of developing ALS among siblings and offspring of those with ALS. In a UK clinic-based study, Hanby et al (2011)\textsuperscript{345} interrogated the pedigrees of over 1500 ALS patients diagnosed over a 15-year period. 0.5% siblings and 1.1% offspring identified had developed ALS. Similarly Fang et al (2009)\textsuperscript{346} used data from the Swedish Multi-Generation Register (1961-2005) and recorded ALS diagnoses in 0.5% siblings of ALS patients. In contrast, only 0.3% offspring developed ALS, although this was significantly higher than the offspring of people without ALS (RR 8.8, 95% CI 6.2 – 12.0). No increased ALS risk was observed among spouses of those with ALS, highlighting the importance of genetic risk factors. One explanation for the difference in risk among offspring between the two studies is the difference in cohorts assessed. Specialist clinics often have a younger cohort of patients who are more likely to have familial disease. Indeed, the UK proband cohort had a mean age of onset of 57.6 years compared with a mean age of diagnosis of 66.1 years among Swedish ALS probands. This is important as the relative risk among siblings and offspring is higher for patients with younger onset disease\textsuperscript{346}.

While both studies noted the absolute risk of developing ALS for relatives increased with increasing age, the relative risk of developing ALS decreased with increasing age of both probands and relatives, a finding which has been mirrored in other neurodegenerative conditions\textsuperscript{346}. Fang et al (2009)\textsuperscript{346} reported that the lower risk of developing ALS among offspring compared with siblings persisted after multiple adjustments for age, sex and follow-up and a higher relative risk for siblings was seen in all separate analyses by age groups. Differential risk for siblings compared with offspring may point to specific genetic
or environmental risk patterns. The finding is interesting as intuitively one would expect any cohort effect to favour an increased incidence of ALS in offspring of patients due to favourable demographic change (increased life expectancy, reduced competing causes of death), better case ascertainment and improvements in diagnostic accuracy with time\textsuperscript{347}. The higher risk in siblings across all age categories argues in favour of shared early environmental exposures with the proband suggesting a potential time dependent gene-environment interaction effect. From a genomic viewpoint, recessive gene action could also explain that apparent cohort effect as siblings have a higher chance of inheriting both recessive alleles from their common parents. While this aligns with higher incidence of ALS in genetically more homogenous populations, there is little else to support this hypothesis.

### 2.3.2. Sex-differentiated risk

Interestingly, Fang et al (2009)\textsuperscript{346} also showed a higher risk of developing ALS among children with a maternal proband (RR 11.7, 95% CI 7.4 – 17.3) compared with children with a paternal proband (RR 6.5, 95% CI 3.7 – 10.3). No sex-specific difference in risk among first-degree relatives was observed. In contrast, Gibson et al (2014)\textsuperscript{348} reported a higher risk of dying due to ALS in male (RR 6.3, 95% CI 3.9 – 9.4) versus female (RR 3.2, 95% CI 1.4 – 5.9) first-degree relatives (p=0.014). In this population-based ALS-mortality study, mothers and fathers of ALS probands had a similar increased risk of ALS, so the apparent increased risk was driven by sex differentiated risk among siblings and children only, with higher relative risks observed for brother versus sisters and sons versus daughters. In interpreting this finding, it is worth remembering that median age of disease onset occurs later in woman compared with men\textsuperscript{349}, a finding which was replicated in this study with a higher mean age of death among female versus male ALS probands\textsuperscript{348}. Correct interpretation of potential sex-differentiated risk profiles among ALS relatives would need to account for this potential source of ascertainment bias. Interestingly, maternal rather than paternal uncles were at highest risk of developing ALS. Together with the observations from Fang et al (2009)\textsuperscript{346} this suggests that genetic risk may be more likely to be transmitted maternally.

This concept can be examined in relation to the liability threshold model\textsuperscript{350}, which describes that the risk of developing a disease is normally distributed in a population, with some individuals being at very high risk and some at very low risk. In this model, one develops a disease once a critical threshold of liability is crossed\textsuperscript{139}. If we consider, that the incidence of ALS is lower among women than men, then the average woman can be considered to be at lower risk of developing ALS than the average man. However, women who develop ALS can be considered to have an exceptionally high-risk profile. If
at least part of this risk is genetic, it makes sense that women with ALS may be more likely to have a higher genetic burden and as such are more likely to transmit this risk to their offspring. This hypothesis could explain the maternal transmission patterns described above.

**2.3.3. Defining familial ALS**

Overall, an increased risk of dying with ALS was observed for second-degree relatives, but not third-, fourth-, or fifth-degree relatives of ALS probands. This is in line with work by Byrne et al (2011), who demonstrated that the risk of a second family member developing ALS increases with increasing kindred size and that more extensive familial clustering of ALS is more likely attributable to direct genetic effects. Among familial ALS kindreds, the prevalence of known ALS-causative mutations is higher among probands with at least two first- or second-degree relatives with ALS compared with ALS probands with distantly related relatives with ALS. Taken together, the significance of familial clustering within a kindred may be determining by models factoring the extent of clustering, family size and the lifetime risk of developing ALS in the general population. This should be used to guide how we define familial ALS in clinical and research practices.

**2.3.4. Neurodegenerative disorders and other conditions**

There is conflicting evidence as to whether there is an increased risk of other neurodegenerative conditions in ALS kindreds. The strongest evidence lies in support of an increased risk of FTD among relatives which may in part be driven by pleiotropic manifestations of rare variants of large effect e.g., C9orf72 repeat expansion. In other population-based studies, various forms of dementia are grouped together which are associated with little or no increased risk among relatives. Relatives of ALS probands were not found to be at increased risk of developing Alzheimer’s dementia or Parkinson’s disease. Separately, no difference in risk of cardiovascular disease, stroke or cancer was observed among relatives of those with ALS compared with controls.

**2.3.5. Neuropsychiatric disease**

Byrne et al (2013) were the first to report on an increased risk of neuropsychiatric disorders among first- and second-degree relatives of patients with ALS. The authors identified that relatives of patients with ALS had a 4-fold increased risk of developing a psychotic illness and 5-fold increased risk of suicide. The risk of neuropsychiatric disease was greatest in relatives from kindreds known to harbour the C9orf72 repeat expansion, but was also present in families without the pathogenic repeat expansion. A second Irish
study\textsuperscript{354} subsequently extended these findings, reporting an increased risk of impulse control disorder, addiction, alcoholism, personality rigidity, and autism spectrum disorder among relatives of those with ALS. These findings were replicated, in part, by Devenney et al (2018)\textsuperscript{355} who also noted an increased risk of schizophrenia, psychotic illness, suicide and autism spectrum disorders among relatives of C9orf72 carriers compared to relatives of non-carriers. A Swedish register-based study\textsuperscript{356} also found an increased risk of psychiatric disorders (encompassing schizophrenia, bipolar disorder, depression, neurotic disorders, stress-related disorders and alcohol and/or drug abuse/dependence) among children of patients with ALS, but not parents or siblings of those with ALS. As discussed above the differences in neuropsychiatric risk in the different relative groups in this study may be a cohort effect owing to the greater recognition of psychiatric disorders in recent times.

2.3.6. Importance

Determining the significance of the co-occurrence of other disorders in ALS kindreds is valuable for identifying novel gene mutations linked to ALS and can be achieved through family aggregation studies as outlined above. An alternative approach is the employment of linkage studies with in-depth phenotypic characterisation. For example, the identification, within one Italian kindred\textsuperscript{357}, of VCP mutations as a common genetic cause for ALS, FTD, inclusion body myopathy and Paget’s disease lead to increased recognition of the importance of the ubiquitination/protein degradation pathway in motor neuron degeneration. Mutant VCP toxicity is partially determined through its impact on TDP-43 protein, a major component of ubiquitin inclusions which is in turn the pathological signature of ALS. The clinical significance of recognising linking “separate” clinical disorders lies in acknowledging the full spectrum of clinical syndromes associated with particular gene mutations. In the case of VCP, prior to broadening the phenotype of Inclusion Body Myopathy, Paget’s disease and Frontotemporal Dementia (IBMPFD) to include ALS, weakness in individuals carrying VCP mutations was routinely ascribed to inclusion-body myopathy only. A diagnosis of ALS in VCP carriers was erroneously believed to be unviable.

While pleiotropic manifestations are a known feature of certain ALS-causative mutations including the C9orf72 repeat expansion, it is probable that they also play a part in the phenotypic expression of polygenic ALS risk. The family aggregation studies identified that the risk of neuropsychiatric disease, particularly psychotic disorders is increased in ALS kindreds. Subsequent analyses of GWAS data from large consortia efforts in ALS and different neuropsychiatric disorders showed evidence of polygenic overlap between ALS and schizophrenia and bipolar disorder\textsuperscript{315, 358}. This work highlights that the study of
apparently unaffected relatives of ALS probands is both feasible and informative and indicates that it may be a mistake to ascribe the entirety of such phenotypic variation within ALS kindreds to known large effects variants like the C9orf72 repeat expansion.

2.4. **Novel Endophenotypic Approaches in ALS**

Identifying what constitutes significant familial clustering, both in the relative extent of disease and the various phenotypic manifestations involved, has important clinical and research implications. From a clinical viewpoint, greater understanding of these points will help guide genetic counselling discussions around the nature of familial disease and the potential risks for unaffected relatives. From a research viewpoint, performing genetic analyses on these informative families, may help us to identify new susceptibility genes and biological pathways in ALS and neuropsychiatric disorders, potentially leading to the development of new and more effective treatments. Furthermore, studying known ALS mutation carriers before they develop symptoms offers the opportunity to enhance our understanding of the natural history of the disease, develop phenotypic prediction models, examine the utility of putative biomarkers and identify the optimal timing for pharmacological intervention. As has been observed recently for children with spinal muscular atrophy (SMA), it is likely that efficacy of new therapeutic agents for ALS will be greater for those treated soon after or before symptom onset.

Finally, while the study of unaffected relatives of those with ALS may enlighten us as to the various phenotypic impact of rare, large-effect and common, small-effect variants (as discussed above), this phenotypic pleiotropy may in itself undermine certain types of genetic analyses. For example, in linkage studies, carriers of the pleiotropic genes who manifest the alternative phenotype may be deemed to be unaffected. This problem necessitates the adoption of alternative phenotypic measures for outcome classification purposes. In this section, I will discuss (i) current limitations in phenotypic modelling of ALS development as it relates to the underlying disease processes, (ii) alternative approaches in modelling gene-phenotype liability and (iii) reflect on what we have learnt from the study of pre-symptomatic gene carriers and how this may be applied the task of identifying novel neuropsychological endophenotypes in ALS.

2.4.1. **Current limitations in phenotypic modelling in ALS**

The study of unaffected relatives of ALS probands is hampered by the use of confusing and often overlapping terminology. The terms asymptomatic, presymptomatic, preclinical and premanifest are commonly used interchangeably to denote unaffected individuals known to carry ALS-causative mutations, who are as such deemed to be at high risk of developing ALS in the future. They are characteristically distinguished from the
symptomatic phase by the lack of overt clinical symptoms or signs and in practice are most commonly recognised in retrospect. “Presymptomatic” and “symptomatic” disease stages reflect an underlying pathobiological continuum, where molecular or cellular (or network) mechanistic dysfunction, transitions from a compensated (“pre-manifest”) to decompensated (“prodromal” or “symptomatic”) state, with the former only observable through the use of biomarkers. Difficulties are inherent in this framework lie in defining when the “true onset” of the ALS disease process occurs, with “transitioning” between different stages is at best considered a semantic distinction, reflecting more on what is observable and when, rather than directly reflecting the disease pathology itself. Furthermore, distinguishing “prodromal disease” from “symptomatic” disease requires consideration of what counts as an unequivocal symptom or sign. In ALS, like with Huntington’s disease, historically motor signs clinically define disease onset, at a cost to the recognition of other cognitive and neuropsychiatric changes which may manifest earlier in the disease process.

2.4.2. Alternative approaches to modelling gene-phenotype liability

While ALS can be considered as a disorder of specific neural networks, this again is a function to some degree of what is measurable. Certainly, structural or functional evidence of network dysfunction reflects the disease pathology in a more direct manner, yet the clinical significance of these changes in an individual is not often clear. Conversely, a high degree of discrepancies exists between clinical diagnoses and pathological results for certain neurodegenerative disorders. In this regard, the concept of “endophenotypes” which is frequently used in psychiatric research may be useful in helping us better identify genetic liability. Endophenotypes are quantitative biological traits which represent intermediate steps in the pathway between genetic burden and clinical disorders. They can be measured at any intermediate level of liability in the cascade from gene to diagnostic syndrome, from measures of protein dysfunction to assessments of neuropsychological function (Figure 2-1).

Endophenotypes are vaulted for the utility in deconstructing complex gene-phenotype pathways. By offering an alternative means to classify phenotypic outcomes more directly correlated with disease liability, they enhance the statistical power of genetic studies. Studies of unaffected relatives allow for determination of heritability of endophenotypes and examination of the relationship between endophenotypes and disease syndrome. The endophenotype should co-segregate with disease within families, with unaffected relatives exhibiting levels of the endophenotype halfway between those of affected individuals and controls. Similarly, endophenotypes should exhibit trait-like properties. They should be present when a disorder is inactive or in
remission and show substantial test-retest reliability over short periods of time. Useful criteria for the identification of putative endophenotypes have been proposed (Figure 2-2).

**Figure 2-1: Endophenotypes as measures of Intermediate Disease Liability.**

*From Cannon and Keller (2006)*

**Endophenotype criteria**

1. The endophenotype is associated with illness in the population.
2. The endophenotype is heritable.
3. The endophenotype is primarily state independent (manifests in an individual whether or not illness is active)
4. Within families, endophenotype and illness co-segregate.
5. The endophenotype found in affected family members is found in non-affected family members at a higher rate than in the general population.

*Figure 2-2: Criteria for defining endophenotypes.*

*From Gottesman and Gould (2003)*
In disorders such as schizophrenia, numerous quantitative neurocognitive and neurophysiological measures have been proposed as potential endophenotypes for the disorder. Neurocognitive endophenotypes include performance on tasks assessing executive function, attention and working memory including Letter-Number Span (LNS), Continuous Performance Task (CPT) and the California Verbal Learning Test (CVLT). Likewise, anti-saccade performance and measures of neurophysiological function including Mismatch Negativity and P50/P300 event related potential amplitude have also been proposed as potential endophenotypes. These endophenotypes have demonstrated both considerable shared heritability with schizophrenia and strong associations with single nucleotide polymorphisms (SNP) of specific candidate schizophrenia risk genes. In this way, these endophenotypes have highlighted the importance of common shared signalling networks and provided insights into the underlying pathobiological mechanisms activated in this disorder.

2.4.3. The study of pre-symptomatic gene carriers in ALS and FTD

2.4.3.1. Studies conducted to date

Currently, the majority of published studies on pre-symptomatic gene carriers in ALS comes from large multi-centre consortia efforts dedicated to exploring this field. Such efforts are required due to the rarity of certain mutations. For example, the prevalence of the pathogenic C9orf72 repeat expansion among healthy adults in the UK is reported as just 0.15%. Consortia such as the Genetic Frontotemporal Dementia Initiative (GENFI) and Advancing Research and Treatment in Frontotemporal Lobar Degeneration (ARTFL) /Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects (LEFFTDS) groups focus on those at genetic risk of developing FTD. The Pre-symptomatic Familial Amyotrophic Lateral Sclerosis (Pre-fALS) study group assess those at genetic of developing ALS, while the Predict to Prevent Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis Study Group (PREV-DEMALS) study follows those at genetic risk for both conditions.

Pre-symptomatic studies in FTD may inform our understanding of the processes underlying cognitive and behavioural changes as develop in ALS, but it is important to recognise that particular pathways affected may be genotype-specific e.g. cerebral white matter hyperintensities which are specific to GRN mutation carriers suggest progranulin plays a role in neuroinflammation. While the PREV-DEMALS study is specifically restricted in this regard, the pathogenic C9orf72 repeat expansion is by default the most commonly explored mutation across all groups, as it is the most common causative mutation for both ALS and FTD.
While larger consortia undoubtedly have an important role to play in studying presymptomatic mutation carriers in rare diseases, a recent systematic review identified that more than half the studies performed assessing pre-symptomatic gene carriers in ALS were smaller, single-centre cohort studies. The vast majority of these studies focused on either presymptomatic SOD1 or C9orf72 repeat expansion carriers. Meta-analysis of the data from these studies is limited by differences in cohort demographics, genotype assessed, study design, assessment methods and comparison groups. Nonetheless identification of some recurring patterns, with respect to pre-morbid clinical features and the evolution of the disease process, is possible. In discussing these findings, the focus is on the features of those carrying the pathogenic C9orf72 repeat expansion, with reference made as relevant to other cohorts, as the repeat expansion is the only notable genetic cause of ALS in Ireland presently identified.

2.4.4. Findings on detailed neuropsychological assessment

2.4.4.1. Asymptomatic FTD cohorts (genotype not differentiated)

An ARTFL/LEFFTDS group study performed detailed longitudinal neuropsychological profiling in 93 mutation carriers (MAPT [31], GRN [28], C9orf72 [34]) and 78 familial non-carriers. Impairments in executive function and processing speed were observed in mutation carriers compared with non-carriers. Executive function, processing speed and verbal fluency also declined more rapidly in mutation carriers, but was not specific to any genotype. An early GENFI group study by Rohrer et al (2015) compared 78 asymptomatic mutation carriers (MAPT [15], GRN [45], C9orf72 [18] mutations) with 102 familial non-carriers. The group adapted an approach previously used in Alzheimer’s research to describe the expected age of onset of an individual. Analysing data from their cohort, they observed a strong correlation between the symptomatic carriers age of onset and the mean age of onset within the family. On this basis, the expected time to onset for an individual was calculated as the mean familial age of onset minus the individual’s age at the time of the assessment i.e., an individual aged 50 at time assessment with a familial mean age of onset would be have an expected time to onset of 5 years. Using this approach, Rohrer et al (2015) observed a time dependent pattern in the development of cognitive and behavioural changes in mutation carriers, with significant differences detected compared to non-carriers 5 years before expected onset in executive function, language, memory and behaviour domains. Tests of immediate recall and verbal fluency tasks only showed differences between mutation carriers and non-carriers was at the time of expected onset. A study by Benussi et al (2019) of 52 asymptomatic mutation carriers (GRN [48], C9orf72 [4]) compared with 73 familial non-carriers replicated these findings with differences detectable between groups on MMSE,
Trail Making Test part A and behaviour 5 years before expected onset. No assessment of the impact of specific genotypes was made. In contrast, a GENFI group study\textsuperscript{375} of 167 symptomatic and pre-symptomatic mutation carriers (C9orf72 [60], GRN [75], MAPT [32]) looked at the impact of genotype on neuropsychiatric symptoms. No differences were observed between the genetic groups with respect to the frequency or severity of neuropsychiatric symptoms (delusions, hallucinations, depression and anxiety). 18/122 (15\%) and 15/122 (12\%) of pre-symptomatic carriers had depression or anxiety, with 1/37 (3\%) presymptomatic C9orf72 carrier experiencing moderately severe hallucinations or delusions. However, no comparison was made with a control population in this study.

2.4.4.2. Asymptomatic C9orf72 carriers

A sub-group analysis by genotype was performed by Rohrer et al (2015)\textsuperscript{372} in the early GENFI study. 18 asymptomatic C9orf72 mutation carriers were compared with 102 familial non-carriers. Significant changes in cognition and behaviour were detectable in C9orf72 carriers up to 20 years before expected disease onset. Behavioural changes were the earliest differences observed occurring 20 years before expected onset. 15 years before expected onset, differences were appreciable on tasks including letter and categorical fluency, digit symbol test, trail making task A and B, logical memory immediate and delayed recall, block design and Boston naming test. Finally, differences on digit span forward and backward and MMSE were appreciable up to 10 years before expected onset. However, these findings were not replicated in a much larger study from the same group\textsuperscript{376} comparing 83 asymptomatic C9orf72 mutation carriers with 249 familial non-carriers. In this study a much less comprehensive neuropsychological assessment was performed and neuropsychological performance was not assessed in relation to expected time to onset. Still, no differences on MMSE or behaviour (CBI-R) were detected between mutation carriers and non-carriers.

Separately, a large prospective, multicentre, observational study (PREV-DEMALS)\textsuperscript{377} examined neuropsychological performance in first-degree relatives of individuals carrying the C9orf72 mutation. 41 asymptomatic mutation carriers were compared with 39 age and sex-matched familial non-carriers. From a comprehensive neuropsychological battery, only praxis scores and total recall scores differentiated between the groups, with mutation carriers performing worse. It is notable however that the mean age of asymptomatic carriers was just below 40 years, with a mean time to expected onset for the group of 25 years. In this regard, the study authors highlight that some neuropsychological changes are evident from an early age among mutation carriers.
A very small but detailed study by Papma et al (2017)\(^\text{378}\) of 18 asymptomatic C9orf72 carriers compared with 15 familial non-carriers observed poorer performance on letter fluency and Stroop colour word interference test among mutation carriers. No participants performed at disorder level (≥ 2 SD below normative data) and the results did not withstand correction for multiple comparisons. In a recent study by Lulé et al (2020)\(^\text{379}\), 36 asymptomatic ALS mutation carriers (C9orf72 [21], SOD1 [15]) were compared with 56 familial non-carriers and 35 healthy controls using a detailed neuropsychological battery. After correction for multiple comparisons, the asymptomatic C9orf72 repeat expansion carriers were found to perform worse on phonemic fluency and visual memory compared to healthy controls. Asymptomatic SOD1 mutation carriers and familial non-carriers demonstrated similar performance to healthy controls. This study also reported on a higher incidence of both depression and anxiety (Hospital Anxiety and Depression Scale [HADS]) among family members of those with ALS (asymptomatic mutation carriers and familial non-carriers) compared to healthy controls. However, among those with family history of ALS, no difference in depression or anxiety were found between groups. Finally, of note, asymptomatic C9orf72 carriers have also been used as controls in other studies measuring disease progression in C9orf72 positive ALS, ALS-FTD or FTD cohorts\(^\text{380, 381}\). The study authors found that letter fluency in asymptomatic C9orf72 carriers was significantly better than C9orf72 positive ALS-FTD or FTD patients at baseline and showed slight improvements over time, which the authors attributed to a practice effect.

### 2.4.5. Structural and functional changes in asymptomatic mutation carriers

#### 2.4.5.1. Asymptomatic FTD cohorts (genotype not differentiated)

Neuroimaging studies have identified widespread changes and distinct genotype-specific imaging patterns among asymptomatic mutation carriers in ALS and FTD. In asymptomatic FTD cohorts where the genotype was not differentiated, degeneration of frontal, temporal, parietal and cerebellar regions have been observed as well as involvement of subcortical structures including the caudate and thalamus\(^\text{359, 382, 383}\). One study from the GENFI group\(^\text{372}\) found that the earliest differences between 78 mutation carriers (MAPT [15], GRN [45], C9orf72 [18]) and 102 familial non-carriers were observed for the insula and temporal lobe (10 years before expected symptom onset). Five years before symptom onset, between group differences were observed for frontal lobe, sub-cortical and whole brain volume, with differences only noted in the parietal lobe and cingulate at expected time of onset. Interestingly, these findings were mirrored by those Benussi et al (2019)\(^\text{374}\) who performed a comprehensive longitudinal study of 52
asymptomatic mutation carriers (GRN [42], C9orf72 [4]) and 73 familial non-carriers, examining clinical, anatomical and neurophysiological measures. The study authors observed, for GRN mutation carriers, changes in GABAergic transmission as evidenced by changes in intracortical facilitation (ICF), short and long-interval intracortical inhibition (SICI/LICI) on transcranial magnetic stimulation (TMS) testing 25-30 years before expected symptom onset. Increased burden of white matter lesion was detected 15 before expected symptom onset with mild cognitive impairment developing 5 years before expected symptom onset (as described above)\(^\text{374}\).

### 2.4.5.2. Asymptomatic C9orf72 carriers

Among asymptomatic C9orf72 carrier cohorts, cross-sectional MRI studies of have also reported atrophy of frontal, temporal parietal and occipital regions\(^\text{359}\), with PET studies demonstrating hypometabolism of frontotemporal, insular, thalamic and basal ganglia regions\(^\text{384}\). The PREV DEMALS study group compared 38 asymptomatic C9orf72 mutation carriers with 29 familial non-carriers and showed extensive white matter track changes in the former including involvement of several frontotemporal related tracts and both corticospinal tracts\(^\text{385}\). Sub-group analysis from GENFI study by Rohrer et al (2015)\(^\text{372}\) of asymptomatic C9orf72 carriers noted the earliest differences detected for carriers were in subcortical regions including the thalamus, the insula, and posterior cortical areas which demonstrated reduced volumes compared with controls 25 years before expected symptom onset. Inter group differences in the cerebellum were noted 10 years before expected onset\(^\text{372}\). A follow-up study from the GENFI consortium\(^\text{376}\), which examined 83 asymptomatic C9orf72 carriers explains the notable atrophy in asymptomatic carriers as occurring secondary to faster cortical thinning linearly throughout adulthood, starting when subjects are in their early 30’s. In contrast to the study by Benussi et al (2019)\(^\text{374}\) described above, no evidence of cortical hyperexcitability was observed in a cross-sectional study comparing asymptomatic C9orf72 mutation carriers with healthy controls\(^\text{386}\), although it is notable that the number of mutation carriers assessed in this study was very small (n=11).

### 2.5. Summary of findings

While the effect sizes are small, a general pattern emerges of predominantly mild executive and verbal fluency impairment among asymptomatic C9orf72 mutation carriers, similar to that seen in symptomatic patient cohorts. The development of changes appears to manifest in a time dependent manner, with changes most appreciable in the years prior to symptom onset. However, changes may be detectable up to decades earlier. These findings are mirrored in the structural and functional
changes observable in asymptomatic mutation carriers. The underlying processes at play drive faster cortical thinning resulting in early widespread degenerative changes among asymptomatic C9orf72 carriers on neuroimaging. While there is a lack of studies examining neurophysiological biomarkers in C9orf72, similar studies in FTD mutation carriers show cortical hyperexcitability precedes structural changes, with cognitive symptoms being last to manifest.

2.6. Limitations of research studies on asymptomatic mutation carriers

2.6.1. Impact of pre-screening

Most data on asymptomatic ALS or FTD carrier cohorts evaluated for this review was obtained from studies assessing the utility of various putative biomarkers in these cohorts. Neuropsychological performance was often not a primary outcome in itself. Instead, these assessments were used most frequently as a pre-screening measure to differentiate symptomatic from asymptomatic gene carriers, with the purpose of the assessment being to ensure that ‘asymptomatic’ participants had no neuropsychological deficits. Alternatively, other studies employed brief global cognitive screening tools to control for the impact of potential confounders across groups, where neuropsychological performance was not the primary outcome. For example, in a study involving 15 asymptomatic C9orf72 repeat expansion carriers, using multimodal MRI to assess pre-morbid salience and sensorimotor network function, all asymptomatic carriers and healthy controls were required to obtain an MMSE score ≥ 27 for inclusion in the study.

The Mini-Mental State Examination (MMSE) is the most frequently applied screening tool used to measure general cognition among asymptomatic ALS and FTD gene mutation carriers. Among the young healthy adult population, to whom asymptomatic mutation carriers presumably belong, the MMSE demonstrates a ceiling effect. Among the ALS population, while its use has been validated, it demonstrates poor sensitivity for detecting clinically significant cognitive changes and certain tasks (e.g., copying, reading aloud) may not be possible for those with significant motor disability. The Montreal Cognitive Assessment (MoCA) screening test, while less commonly used for cognitive screening purposes, also shares these limitations as a screening tool in both cohorts and its use has not yet been validated among patients with ALS. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) tool is a highly sensitive brief screening measure specifically developed for patients with ALS, where the utility of other cognitive screening may be comprised due to the patient’s motor disability. It is designed to distinguish
ALS-specific cognitive and behavioural changes from changes more likely attributable to other disorders. The advantage of this tool is that it allows standardised screening across symptomatic and asymptomatic ALS gene carrier cohorts. Yet, while certain mutations (e.g., C9orf72 repeat expansion) are common to both ALS and FTD, this tool has not been widely adopted among FTD cohorts owing to its origins in ALS research. To date, its utility as a screening tool among asymptomatic ALS gene mutation carriers is unclear. Two small studies showed no difference in ECAS total or sub-scores between asymptomatic C9orf72 carriers and familial non-carriers or healthy controls, where each study assessed 21 and 16 asymptomatic carriers respectively. Among ALS cohorts, the Beaumont Behavioural Index (BBI) showed the best potential for detecting mild impairment in a recent review of behavioural screening tools. As with ECAS, its use has not yet been widely in FTD research. No studies were identified where it used to screen asymptomatic ALS or FTD gene mutation carriers. While the use of brief cognitive and behavioural screening tools would be preferrable for both research and clinical purposes, deficits in asymptomatic gene carriers are likely to be subtle and so comprehensive detailed neuropsychological assessments are warranted until such point that these measures can be refined.

2.6.2. Familial non-carriers as controls

Familial non-carriers are commonly used as controls in studies of asymptomatic gene carriers. When the primary outcome is to discover the clinical effects of a specific genotype, this is certainly an acceptable approach which diminishes the impact of other shared familial risks, be they environmental or genetic. Yet an opportunity is lost by not comparing non-mutation carriers to an otherwise healthy control population. ALS is a known oligogenic disorder. Within a kindred, both known ALS-gene carriers and non-carriers may harbour other gene variants necessary for disease development or expression. This is demonstrated in work by Byrne et al (2013) which demonstrated an increased risk of psychotic and suicide in relatives of C9orf72 positive ALS patients, but noted this increased risk was also present to a lesser extent in families of non-carriers compared with control families. In this regard, prospectively controlling for such variants may be an overly simplistic approach, which while enhancing the power in examining a specific genotype, reneges on the opportunity to delineate the impact of important gene-gene or gene-environment interactions.

2.6.3. Determining time to expected onset in ALS

The approach adopted by Rohrer et al (2015) for examining changes in asymptomatic gene carriers in relation to their “expected time of onset” has been widely adopted and
in many respects has been informative in enhancing our understanding of how various FTD phenotypes develop. From an ALS viewpoint, the question now arises as to whether a similar approach could be adopted in the study of asymptomatic ALS gene mutation carriers, and what that approach would it look like? Rohrer’s approach was informed by familial Alzheimer’s disease literature where a strong relationship was evident between both the parental age and mean age at onset within a family and an individual’s age at symptom onset\textsuperscript{373}. Rohrer’s study in an FTD population found a similar correlation between an individual’s age of onset and the mean age of onset of FTD within a family, but no correlation was found with parental age of onset. Rohrer’s approach has several limitations with respect to the study of ALS gene mutation carriers. Firstly, it is dependent on familial clustering of the disease which is not always evident\textsuperscript{250}. Secondly, a lot of cases of familial clustering are accounted for by parent-offspring transmission. Finally, with respect to ALS, the definition of disease onset relates to the onset of motor symptoms. At present it remains unclear as to how this correlates with the onset of neuropsychological symptoms. Indeed, not all ALS patients will manifest cognitive or behavioural changes\textsuperscript{16} and factors underlying cognitive reserve may separately impact on when and if such changes develop\textsuperscript{396}. Further work is required to develop an “expected time to onset” model appropriate for the study of development of neuropsychological changes among unaffected relatives of ALS probands.

### 2.7. Lessons in the search for novel neuropsychological endophenotypes in ALS

The study of asymptomatic gene carriers in ALS and FTD has revealed that subtle cognitive deficits, mainly executive, are detectable at an early stage and may evolve as part of the underlying disease processes. Comprehensive neuropsychological assessments are required to detect these changes, and should also explore potential behavioural and neuropsychiatric manifestations which are understudied. Family controls enhance power when studying the effects of specific genotypes. Yet as disease processes and neural networks impacted may be genotype-specific, it is not clear that extrapolation of findings from asymptomatic studies to the wider “at risk” population is valid in all cases. Asymptomatic gene carriers, while of interest, are not the only individuals at genetic risk of developing ALS. No single gene of large effect can be identified as a cause for nearly 30% of familial ALS patients, rising to over 90% for sporadic cases\textsuperscript{248}, meaning the genetic underpinnings of the disease, for the vast majority of people with ALS, remains enigmatic.
All unaffected relatives also share degree of genetic risk with people with ALS. This shared genetic risk underpins studies of ALS heritability and can be used to expand our understanding of the intersection between ALS and other disorders. As with schizophrenia, neurocognitive deficits such as executive dysfunction may represent potential endophenotypes for ALS, linking the condition with other neuropsychiatric disorders. This aligns with the endophenotype model proposed by Cannon and Keller (2006) where it is expected that a single endophenotype should contribute to multiple genetically-linked disorders. A recent GWAS study a 14% polygenic overlap between ALS and schizophrenia, with enriching of both cohorts for NCKAP5L loci noted. Information processing speed performance has also been associated with this locus, suggesting this measure could be an endophenotypic bridge connecting both disorders, as well as linking to bipolar disorder and autism. Conversely, the broad range of cognitive and behavioural deficits seen in patients with ALS suggest it is likely that multiple endophenotypes will be identified that will link to the clinical syndrome of ALS. However, further work will be required to determine whether any putative neuropsychological endophenotype manifests in a trait or state manner, with the latter reflecting a measure of disease activity and former of greater utility in identifying novel ALS associated genes.

2.8. Future Directions

In this chapter, I applied a “clan genomics” model in consideration to ALS genetic risk. It is clear from my review that both population-specific common variants and rare, larger effect familial variants influence ALS risk and its phenotypic manifestations. Indeed, the current understanding of the genetic architecture of ALS and where the “hidden heritability” lies would suggest that there is much to learn from performing deep-phenotyping studies in ‘high genetic risk’ ALS kindreds, particularly those significant clustering of other neuropsychiatric disorders.

As such, there is a pressing need now to identify quantifiable traits (endophenotypes) in unaffected relatives of those with ALS. Such endophenotypes could be exploited to enhance the statistical power of genetic studies and facilitate the discovery of the common pathobiological underlying shared disease risk within such kindreds.

Yet, encompassing the concept of endophenotypes in ALS research may also compel a shift in perspective. For many, the optimum approach to managing heterogeneity in ALS is to strive for dissection of the disorder into sub-phenotypes. Future classification systems may instead recategorize conditions as profiles of multi-endophenotypic traits
and their associated genetic determinants which may be targeted separately for therapeutic purposes.
3. Chapter 3: Thesis Hypotheses, Aims & Objectives

3.1. Introduction

Considerable work has already been done investigating how neuropsychological and neuropsychiatric changes observed in patients with ALS may represent separate sub-phenotypes thought to arise from the degeneration of distinct neural networks. Equally, analyses of pathways implicated by known ALS gene mutations has yielded novel insights into pathogenesis of ALS. Yet, exploiting distinct sub-phenotypes to identify new ALS-causative genes has not been possible in the majority of cases as the infrequent occurrence of the disease, even in cases of familial clustering, results in inadequate power to determine meaningful outcomes in linkage studies. Neuropsychological endophenotypes which represent simple traits, as distinct from clinical syndromes, may offer utility as clinical biomarkers, helping to increase the statistical power of genetic studies as they are genetically simpler and closer to the level of gene action. The detection of such traits through the study of unaffected relatives of those with ALS, offers the opportunity to bridge the gap in our understanding of phenotype-genotype correlations and affected pathways in ALS with potential to identify novel targets for drug development.

3.2. The Primary Objective

The primary working hypothesis of this study is that relatives of patients with ALS exhibit cognitive, behavioural and psychiatric changes which reflect a shared increased genetic risk between ALS and other neuropsychiatric disorders.

This study affords insight into the concept of genetic risk in ALS through the novel direct characterisation of the neuropsychiatric, neuropsychological and behavioural phenotype within ALS kindreds. In turn, this work will advance our understanding of common shared biological pathways impaired in ALS and associated neuropsychiatric disorders, which in turn may lead to the discovery of potential novel therapeutic targets. In order to test this multifaceted hypothesis, several aims for this project were established. These are outlined below.
3.3. **Aim 1: To examine the impact of population-specific genetic signatures on ALS risk and phenotype**

Complete consideration of genetic risk accounts for variants inherited from both distant ancestors (common, small effect) and more recent ancestors (rare, larger effect). In ALS, significant geographical heterogeneity in disease incidence and phenotype is reported. Yet, how much of the variation in risk and phenotype is determined by population-specific genetic signatures remains to be determined.

### 3.3.1. Specific aims:

1. To estimate the heritability of ALS in a well characterised population
2. To examine the impact of ALS genetic signatures on phenotypic manifestations across different populations

#### 3.3.1.1. Rationale and approach:

The extent to which the variation in ALS risk is attributable to genetic factors is explained by the heritability of the disorder. This has never been estimated before in the genetically homogenous Irish population. Furthermore, previous heritability estimates were limited by relying on reported ALS prevalence data, rather than direct estimates of population disease incidence. The influence of population genetic signatures can also be assessed by examining phenotypic variation across populations of different ancestral origin. To date, little is known about the demographic and clinical characteristics of ALS in admixed Latin-American populations and how this may be influenced the population genetic signature as explained by known ALS-associated variants.

3.4. **Aim 2: To identify which ALS kindreds carry the greatest burden of shared genetic risk between ALS and other neuropsychiatric disorders.**

The increased incidence of neuropsychiatric disorders in ALS kindreds may arise from the unique combinations of rare variants characteristic of a recent family lineage. Our current conceptualisation of “familial ALS” does not account for these expanded phenotypic manifestations. Recognition of the full spectrum of familial disease is important to aid in identification and prioritisation of informative ALS families for genetic studies.

3.4.1. **Specific aims:**

1. To evaluate the impact of current familial ALS classification criteria
2. To evaluate the impact of recently identified genetic and phenotypic representations of ALS on familial ALS incidence
3. To distinguish when the occurrence of clustering of clinical disorders within families exceeds that expected by chance alone
4. To examine the clinical relevance of both classical and expanded forms of familial ALS

3.4.1.1. **Rationale and approach:**

Since the Byrne criteria for familial ALS were published, our understanding of what constitutes familial disease has expanded to encompass numerous neuropsychiatric disorders. In identifying and prioritising ALS kindreds for genetic analyses, there is a need to review what we have learnt from these criteria, examine how and if they should be expanded to include these phenotypic manifestations and explore the clinical impact of any such expansion in what is considered as familial disease.

and the clinical features of both classical and expanded forms of familial ALS are contrasted.

3.5. **Aim 3: To characterise the sub-clinical phenotype of those at increased risk of developing ALS**

Several studies have identified an increased incidence of neuropsychiatric disorders among ALS kindreds, through proxy-report measures. Yet few studies have directly assessed large numbers of unaffected relatives of people with ALS to characterise and quantify the occurrence of sub-clinical cognitive, behavioural or psychiatric disturbance. Furthermore, little is known about how such diverging phenotypic manifestations may be impacted by familial clustering of disease or known gene variants.

3.5.1. **Specific aims:**

1. To identify whether common cognitive, behavioural and neuropsychiatric signatures cluster with increased frequency in relatives of ALS patients using direct neuropsychological and neuropsychiatric assessments of first and second-degree relatives of patients with ALS compared with controls

2. To determine if changes observed occur equally across all families or if there is evidence of clustering within certain ALS kindreds

3. To assess the impact of the C9orf72 repeat expansion on objective (1) and (2)

4. Where possible, characterize the impact of 1) possible expansion from one generation to the next and 2) variable inheritance patterns, including oligogenic models on variable phenotypic manifestations among C9orf72 carriers

3.5.1.1. **Rationale and approach:**

Neuropsychiatric disorders occur frequently in relatives of people with ALS, not specifically limited to families where the pathogenic C9orf72 repeat expansion has been detected. This is the first study to directly assess and deep phenotype a large number of unaffected first and second-degree relatives of people with ALS, in families with clustering of neuropsychiatric conditions. Recognition of shared neuropsychological endophenotypes will implicate dysfunction in certain neural networks, with potential to highlight novel ALS-associated variants, distinct from the pathogenic C9orf72 repeat expansion.
The specific aims are achieved by designing and carrying out a case-control deep phenotyping study. The cognitive, behavioural, neuropsychiatric and genetic profiles of first- and second-degree relatives of people with ALS are compared against those of healthy controls. ALS families are recruited through a representative portion of incident ALS patients identified using the Irish ALS Register.
4. Chapter 4: Methodology

4.1. General resources

4.1.1. Irish ALS Register

An Irish population-based register for patients with ALS has been in operation since 1994\textsuperscript{264, 400, 401}. The vast majority of ALS cases, included on the Irish ALS Register, are identified through the Irish National ALS Clinic at Beaumont Hospital. Complete case ascertainment is ensured through employment of multiple sources including consultant neurologists, neurophysiologists, neuropathologists, neurosurgeons, primary care physicians, and the Irish Motor Neuron Disease Association\textsuperscript{400}. All individuals confirmed to have possible, probable or definite ALS according to El Escorial criteria\textsuperscript{4} are appropriately consented and enrolled on the Register. People with motor neuron diseases other than ALS (including PLS, PMA and Kennedy’s disease) are also included on the Register, with children aged 14 years or less excluded due to clinical overlap with paediatric forms of motor neuron disease\textsuperscript{400}.

Following confirmation of diagnosis of motor neuron disease, patients consent to completion of a semi-structured telephone interview using a standardized questionnaire and regular review of their clinical records, where possible. Data collected includes information on residency, ALS clinical features, medical care provided and outcomes, as well as information on the individual’s family size and structure. All informants are asked specifically about the occurrence of any neurodegenerative or neuropsychiatric conditions among their first and second-degree relatives. A diagnosis of FTD in a relative is accepted if 1) they were diagnosed by a physician with expertise in cognitive disorders or 2) the description of the relative was deemed to meet Neary criteria\textsuperscript{15} by a neurologist experienced in diagnosing patients with FTD. For those identified posthumously, a direct chart review and interview with a family member is conducted where possible. The Irish ALS Register is maintained at the Academic Unit of Neurology, Trinity College Dublin.

4.1.2. Irish ALS DNA Biobank

The ALS DNA biobank was established in 1999 to allow for collection and storage of DNA samples (extracted from venous leucocytes) from patients registered with the Irish ALS Register. A control DNA biobank of samples collected from phenotypically normal population-matched controls is available for comparison purposes. All DNA samples are available for research purposes only. As such, the person donating their DNA sample is
not aware of the results of any genomic analysis performed as it may pertain to them as an individual. Equally, researchers do not have access to personal identifiers corresponding to DNA samples. Genomic analysis is performed in-house in the Smurfit Institute of Genetics, Trinity College Dublin.

All DNA samples are tested for established high-penetrance ALS-associated variants\(^{145,264}\) and stored to allow for retrospective assessment of new genes. Through the use of these samples, in conjunction with data from the Irish ALS Register, the clinical phenotype of novel gene discoveries has been characterised\(^{264}\). In addition, patient and control DNA samples have been used to identify novel ALS-associated variants as part of larger international ALS consortium efforts (Project MinE, www.projectmine.com\(^{402}\).

4.1.2.1. C9orf72 repeat expansion

All patients are screened for the presence of the pathogenic GGGGCC hexanucleotide repeat expansion in C9orf72 by repeat-primed PCR, with amplicon fragment length analysis\(^{141}\). This methodology has previously been validated with positive and negative controls using Southern Blotting\(^{264}\). Amplified fragments were measured by capillary electrophoresis on an Applied Biosystems 3500 Series Genetic Analyzer and visualized using Gene Mapper software version 4.0.

Patients with 30 hexanucleotide repeats or above were deemed positive for the expansion, as this is the threshold most frequently used to define a pathogenic expansion and so allows for comparisons to be made between our data and that from other studies. Expansions of between 20-29 repeats were categorized as intermediate length expansions as there is some evidence of a possible association between expansion lengths in this range and neuropsychiatric symptoms\(^{275}\). Finally, those with repeat lengths of less than 19 were deemed negative for the expansion. The vast majority of Irish ALS DNA samples deemed negative for the C9orf72 repeat expansion carried less than 10 repeats, with the most frequently observed repeat length of 1-2 units\(^{275}\).

4.1.2.2. Genomic Analysis

Next-generation DNA sequencing is carried out through Illumina paired-end, PCR free, whole genome sequencing and Illumina paired-end, target-enriched sequencing on DNA samples from patient and control databases. Participants are screened for exonic and splice-site variants (Single Nucleotide Polymorphisms (SNPs) and Insertions/Deletions (INDELs)) in the exons of 30 genes considered to be linked to ALS (ALS2, ANG,
CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERBB4, FIG4, FUS, hnRNPA1, LMNB1, MATR3, NEFH, OPTN, PFN1, PRPH, SETX, SIGMAR1, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TBK1, UBQLN2, UNC13A, VAPB, VCP).

To screen for high-penetrance, variants which are present in any controls or at a maximum allele frequency (MAF) exceeding 0.05 in reference population datasets are filtered. Variants which are present in the ALS online genetics database (ALSoD)\textsuperscript{403} or the Human Gene Mutation Database V.2017.2 (HGMD)\textsuperscript{404} and are reported in the literature as being familial or highly-penetrant were considered to be Mendelian causes of ALS. The pathogenicity of putative variants is assessed in accordance with the American College of Medical Genetics (ACMG) guidelines\textsuperscript{405}. Variants are classified as “pathogenic”, “likely pathogenic”, “uncertain significance”, “likely benign” or “benign” based on the burden of evidence from various data sources (e.g., population, computational, functional and segregation data). Variants which are not present in any population reference dataset or reported in the Human Gene Mutation Database v.2017.2 (HGMD)\textsuperscript{404} are considered to be novel mutations.

To allow for assessment of oligogenic inheritance patterns, all individuals with ALS with co-occurrence of known or putative ALS genes variants are identified. A targeted high-throughput sequencing study by Kenna et al (2013)\textsuperscript{145} identified that 1.6% of Irish ALS patients carried multiple known/putative ALS gene variants, including both TARDBP mutation carriers identified. Finally, the extent of genetic heterogeneity across populations is determined by comparing the estimated frequencies of known or putative ALS gene variants among the Irish patient population with those observed in other populations. For example, in comparison with Italian ALS patients, Irish patients are twice as likely to carry the pathogenic C9orf72 repeat expansion. In contrast, Italian patients are more likely to carry SOD1 or TARDBP gene mutations\textsuperscript{145}.

\textit{4.1.3. Departmental studies}

\textit{4.1.3.1. Comprehensive neuropsychological assessment in ALS patients}

Data is available from several population-based, case-control, cross-sectional and longitudinal studies, assessing the incidence and profile of neuropsychological changes in ALS patients\textsuperscript{177, 406, 407}. Patients for these studies were recruited through the Irish ALS Register from 2006 onwards. Age, gender and education matched controls were recruited through a volunteer network. Those reporting a history of any neurological condition that could impact cognition, prior learning disability or specific learning
difficulties, severe active mental illness or use of psychoactive medications and non-native English speakers were excluded.

Serial clinical and neuropsychological assessments were performed at the participants' home at regular intervals. Study participation was discontinued based on accrued physical disability or death in patient or if the participant expressed a desire to discontinue. Details of the neuropsychological tasks performed are described in supplementary materials (Appendix 1). Information on participant behaviour was obtained through direct evaluation of the patient and where possible completion of caregiver completed behavioural assessment questionnaires. Raw and normative data are available for each task. In addition, cognitive and behavioural categorisations for all ALS patients are reported based on various consensus criteria\textsuperscript{180, 196, 197}.

4.1.3.2. Cognitive screening in ALS patients

Edinburgh Cognitive and Behavioural ALS Screen (ECAS) data are available for over 300 ALS patients and 250 healthy controls. Patients were approached and tested on the ECAS at the Irish National ALS Clinic in Beaumont Hospital at their first clinic visit and at regular intervals thereafter. Controls were recruited through a volunteer network and assessed at home as part of the larger population-based neuropsychological assessments outlined above. Patient and control participants were subject to the same exclusion criteria pertaining to those participating in detailed neuropsychological assessment studies outlined above. The ECAS screening tool has previously been validated in the Irish population and Irish age- and education-adjusted normative data generated\textsuperscript{408}.

4.1.3.3. Irish ALS family aggregation studies

Two population-based, case-control families aggregation studies were performed running sequentially from 2008 – 2014\textsuperscript{314, 354}. The first study focused equally on medical and psychiatric conditions within ALS kindreds. The second study (based on the results of the initial study) expanded the assessment of neuropsychiatric disorders within families. The study procedures were as follows.

ALS patients were recruited through the Irish ALS Register and age and gender matched controls recruited through a volunteer network. Only one ALS patient was recruited per family to participate in the studies. All participants required the availability of a reliable relative for corroboration purposes. Participants enrolled in the studies were asked to complete a validated questionnaire followed by a standardised semi-structured interview
(via telephone or face-to-face) with the researcher. Participants were asked to provide demographic and medical information on all first and second-degree relatives including name, age, all medical conditions and if deceased, age at death and date, place and cause of death.

During the semi-structured interview, participants were asked directly about the occurrence of ALS, FTD, dementia, Parkinson's disease, schizophrenia or other psychotic illness, suicide, depression, anxiety or any other neurological, neurodegenerative or neuropsychiatric disorders among relatives. To increase the validity of the data, the family history information was verified by at one other family member. In addition, researchers obtained death certificates for all deceased family members and in cases where a family history of ALS was reported, a review of the medical and direction examination of the affected relative was performed, where possible. Detailed pedigrees were constructed for all participating kindreds. Data from Irish censuses (1901 and 1911) was used to refine genealogical information as required.

4.2. Project Specific Study Methodologies

4.2.1. Creation of Irish Familial ALS Database

4.2.1.1. Identification of Irish Familial ALS families

Data from the Irish ALS Register from 1994 to 2020 were interrogated. All cases reporting a history of suspected or confirmed ALS or FTD in at least one relative were collated. Where feasible, genealogical records obtained through the family aggregation studies were reviewed. The DNA database was cross-referenced with the clinical database and the genetic status was determined in all cases for whom DNA was available. Additional individuals with a known gene variant (identified through Irish ALS genomic studies\textsuperscript{145}), not previously identified by family history of ALS or FTD, were collated. Detailed pedigrees were constructed for all potential familial ALS cases and modes of inheritance examined.

4.2.1.2. Exclusion criteria

Cases with a diagnosis of Kennedy's disease or PLS were excluded. Individuals who had a family history suspicious for ALS (e.g. relative died from "muscle wasting disease"), in whom we could not confirm the diagnosis, were excluded. Similarly, individuals with a relative with dementia, in whom the nature of the dementia could not be accurately
determined, were excluded. Individuals with an intermediate length C9orf72 repeat expansion and no family history of ALS or FTD were excluded.

4.2.1.3. Diagnostic criteria for Familial ALS

The presence and classification of Familial ALS was determined using our previously defined criteria (Figure 4-1)\(^{246}\).

<table>
<thead>
<tr>
<th>Byrne criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definite FALS:</strong> An ALS patient with at least two first- or second-degree relatives with ALS or an ALS patient with at least one relative with ALS and gene-positive cosegregation.</td>
</tr>
<tr>
<td><strong>Probable FALS:</strong> An ALS patient with one first- or second-degree relative with ALS.</td>
</tr>
<tr>
<td><strong>Possible FALS:</strong> An ALS patient with a distant relative with ALS or Sporadic ALS patient, but positive for a FALS gene or an ALS patient with a family member with confirmed frontotemporal dementia</td>
</tr>
</tbody>
</table>

*Figure 4-1: Byrne criteria for familial amyotrophic lateral sclerosis (FALS).*

4.2.2. Irish Family Aggregation Study 2015 – 2017

An observational, population-based case-control family aggregation study was performed for all ALS incident cases diagnosed between 1\(^{st}\) January 2015 to 31\(^{st}\) December 2017. The recruitment procedures for ALS patients and healthy controls were the same as per the previous Irish family aggregation studies. ALS patients with definite, probable or possible ALS by El Escorial criteria\(^4\) were recruited through the Irish ALS register with age and gender matched controls recruited through a volunteer network. Patients and controls who were unable to provide detail of their family’s medical history (e.g., cases of adoption) were excluded. Likewise, patients who had a family member with ALS who participated in one of the previous Irish family aggregation studies were approached for this study and the recorded family history was updated. However, in the combined analysis, only the family history provided by one ALS proband within a kindred was included.
All participants completed a validated Family History Questionnaire and semi-structured interview as previously described to assess the presence of neurodegenerative and neuropsychiatric disorders among their relatives. Demographic data collected on the informer (ALS proband or control) included (1) gender, (2) age and (3) highest educational attainment. Demographic data collected about relatives included (1) gender, (2) age, (3) relationship to proband, (4) maternal or paternal relative, (5) living or deceased and (6) age and cause of death if deceased. Participants provided medical and psychiatric information on all first- and second-degree relatives. Specifically, they were asked to report on the occurrence of ALS, FTD, dementia (unspecified), Alzheimer’s dementia, vascular dementia, multiple sclerosis, Huntington’s disease, Parkinson’s disease, stroke, epilepsy, Tourette’s syndrome, depression, anxiety, bipolar affective disorder, suicide, social anxiety, post traumatic stress disorder, obsessive-compulsive disorder, phobias, addictions including alcohol and drugs, schizophrenia, psychosis, eating disorders, personality disorders, autism spectrum disorders, learning disabilities, attention deficit hyperactivity disorder, dyslexia and other unspecified neuropsychiatric disorders.

The semi-structured interview provided the opportunity to review all the data provided in the family history questionnaire. Any missing information was addressed. The nature of any reported diagnoses was explored using a specified list of questions to ensure diagnoses met DSM IV criteria. In all cases, the family history information provided was verified by at least one other family member. The validity of this approach has been verified against death certifications in the previous family aggregation studies.

A meta-analysis was conducted using available data from all three family aggregation studies. As the family history questionnaire was more comprehensive with respect to neuropsychiatric disorders for the latter two studies, compilation of psychiatric variables was not possible in all cases across all three studies. Data analysis procedures and results are reported in Chapter 8.

### 4.3. Irish ALS Pleiotropy Study

#### 4.3.1. Study design

This was a prospective, cross-sectional, observational, population-based case-control study of cognitive, behavioural and neuropsychiatric traits in first- and second-degree relatives of ALS probands compared with healthy controls. The majority of study visits were conducted at the participants home, although occasionally other quiet
environments more convenient for the participant were used. Data gathered included demographic, neuropsychological, neuropsychiatric and genetic data. The procedures for data collection and analysis are described in more detail below. This study was conducted with support of Professor Sharon Abrahams and her team in the University of Edinburgh.

4.3.2. Participant recruitment

4.3.2.1. Relative recruitment

Relatives of ALS patients who had previously participated in a population-based case control study of neuropsychological function in ALS (1st January 2015 to 31st December 2017)\textsuperscript{177} were approached to participate in the study. This approach was chosen to allow for comparison of data between ALS patients and their relatives. A second cohort of relatives of patients with familial ALS diagnosed between 1st January 2008 and 31st December 2019 were also approached. As familial ALS cases account for between 5-20% of all ALS patients, this expanded timeline was chosen to allow for sufficient recruitment of relatives from families with familial clustering of ALS. Good relations are maintained with many families or next-of-kin of ALS patients, even after the patient is deceased, with many family members wishing to support ongoing research. In some cases where the person with ALS had died, the family members were approached directly. No families were approached who had not previously expressed interest in directly engaging with research efforts.

An information leaflet detailing the study rationale and procedures was provided to ALS patients, for dissemination within their kindred, or given directly to relatives as applicable. Potential participants were contacted a minimum of 1 week later to allow for sufficient time to review the study information. At this point, potential participants had the opportunity to address any questions or concerns they had. If they decided to participate in the project, a home visit was scheduled. Alternatively, study visits could be conducted at an alternative location suggested by the participant (e.g., workplace) if a suitable quiet environment was available or in an office in the Trinity Biomedical Sciences Institute, as best suited the study participant. In cases where individuals declined to participate in the project, their reason for the same, if stated, was documented.

4.3.2.2. Healthy control recruitment

Age-, sex- and education-matched controls were recruited through a volunteer network established by the Academic Unit of Neurology, Trinity College Dublin. Potential controls
were informed about the research project and sent the study information leaflet if they expressed an interest in participating. A follow-up phone call was made a minimum of 1 week later to answer any questions they had pertaining to the study. For those interested in participating, study visits were scheduled to be conducted at home or an alternative quiet environment that suited the participant. Controls underwent the same process of assessment as relatives.

4.3.3. Case Ascertainment, Inclusion and Exclusion Criteria

Study recruitment was supervised by Senior Consultant Neurologist (OH) and Principal Clinical Neuropsychologist (NP). The inclusion and exclusion criteria for the research project are detailed below. Inclusion criteria were different for relatives and healthy controls. The same exclusion criteria applied for both groups. In any case, where exclusion criteria were identified during the assessment (e.g., cognitive testing suggestive of dyslexia), data collection was completed as feasible but excluded from analysis. The final decision to exclude any potential participant was decided by the study supervisor (OH).

4.3.3.1. Inclusion criteria

Relatives

☑ Relative of incident Irish ALS patient diagnosed January 2015 to December 2017 and/or relative of Irish familial ALS patient diagnosed January 2008 to December 2019.

☑ Age 18 years or older.

Healthy controls

☑ No family history of MND and/or FTD.

☑ Age 18 years or older.

Exclusion criteria (for relatives and healthy controls)

☐ Symptoms or signs in keeping with a diagnosis of MND and/or FTD.

☐ Any neurologic, psychiatric or medical conditions affecting cognitive, behavioural or neuropsychiatric status.
Any learning disability or developmental disorder.

Alcohol dependence syndrome.

Severe active mental illness.

Current use of high-dose psychoactive medication that affect one’s ability to engage in neuropsychological and neuropsychiatric testing.

Non-native English speaker.

Inability to read or write.

Inability to give informed consent.

4.3.4. Study Procedures

4.3.4.1. Demographic Data

A brief semi-structured interview was completed to collect demographic variables on all study participants including: (1) date of birth, (2) gender, (3) handedness, (4) occupation and employment status, (5) years of education and age at cessation of formal education, (6) marital status, (7) list of any medications used, (8) vascular risk factors (hypertension, hypercholesterolaemia and diabetes mellitus), (9) head trauma and (10) heavy metal exposure. Participants were also re-screened to ensure they met inclusion and exclusion criteria for the study.

4.3.4.2. Neuropsychological Assessment

Cognitive and behavioural function in relatives and healthy controls was assessed using a comprehensive neuropsychological battery (Table 4-1 A). The domains of executive function, memory, language and visuospatial ability were all assessed, with details of the specific tasks used described in the sections below.

The neuropsychological assessment was administered by trained researchers, with regular assessments of inter-rater reliability performed. The assessment was performed in a quiet environment, free from any distractions (usually the participants' home). The neuropsychological battery was carried out in a fixed order, with the whole battery taking approximate 2 hours to complete. To minimise the impact of fatigue, participants were made aware that they could take a short break after completion of any task, except during
memory tasks (inclusive of the 30-minute interval between registration and delayed recall).

4.3.4.3. Neuropsychiatric Assessment

Neuropsychiatric function was assessed using a detailed battery of neuropsychiatric tests which were administered online using Qualtrics (Table 4-1 B). Participants were screened for sub-clinical neuropsychiatric traits including affective and psychotic disturbance, alcohol abuse, obsessive and compulsive behaviours, impulsiveness and apathy among others. All participants were emailed a link to the portal with their unique identifier code. Participants were advised to complete the assessment independently, working in a distraction free environment. The battery takes approximately 20 minutes to complete, with participants allowed to take breaks and resume the battery as they wish. Participants who had not accessed the portal or completed all questionnaires were sent a reminder email two weeks later.

4.3.4.4. Genetic data

A peripheral venous blood sample was collected, during the study visit, for all participants for DNA extraction and analysis. All samples collected were coded at source and stored in the Smurfit Institute of Genetics, Trinity College Dublin where genetic analysis was performed. Participants and researchers collecting the DNA samples were not informed of individual genetic test results. Equally, researchers performing the genetic testing were not privy to personal identifiers for the study participant.

All DNA samples were tested for the presence of the pathogenic C9orf72 repeat expansion, using the same approaches and cut-offs as per patient DNA samples. DNA samples from relatives of ALS probands are stored indefinitely as part of the novel Irish ALS Relative DNA Biobank. Explicit consent is obtained for this purpose during the informed consent process. Additional genomic analysis has not been performed on any relative DNA samples as part of this project but it is hoped that the results of the neuropsychological and neuropsychiatric assessments performed in this study will help identify informative ALS kindreds with clustering of neuropsychiatric disease to prioritise for future genomic studies. Control DNA samples are stored as part of the Irish ALS Control DNA Biobank for comparison purposes in future ALS genomic studies.
## Table 4-1: Neuropsychological and neuropsychiatric assessment tools

### A: Neuropsychological assessment

<table>
<thead>
<tr>
<th>Assessment tool</th>
<th>Cognitive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive screening tool</td>
<td></td>
</tr>
<tr>
<td>**Edinburgh Cognitive and Behavioural Screen (ECAS)**493</td>
<td>Measures language, verbal fluency, executive functioning, memory and visuospatial abilities</td>
</tr>
<tr>
<td><strong>Current/pre-morbid intellectual functioning</strong></td>
<td></td>
</tr>
<tr>
<td>**Test of Premorbid Function UK (TOPF-UK)**409</td>
<td>Premorbid Intellectual Functioning</td>
</tr>
<tr>
<td>**Wechsler Abbreviated Scale of Intelligence 2nd Version (WASI-II)**410</td>
<td><strong>Vocabulary</strong> Measures semantic knowledge, verbal comprehension and expression <strong>Matrix Reasoning</strong> Measures nonverbal abstract problem solving and inductive reasoning</td>
</tr>
<tr>
<td><strong>Executive functioning</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Verbal Fluency</strong></td>
<td>A series of lexical and semantic fluency tasks with restrictive metrics</td>
</tr>
<tr>
<td><strong>Colour-Word Interference Test D-KEFS</strong>411</td>
<td>Colour naming Measures multiple dimensions of executive control, including inhibitory control, error monitoring, selective attention and cognitive flexibility. This is also a measure of speed processing</td>
</tr>
<tr>
<td>**Digit Span (WAIS-IV)**312</td>
<td>Forward Measures attention and working memory</td>
</tr>
<tr>
<td>**Sustained Attention to Response Task (SART)**413</td>
<td>Measures sustained attention ability while withholding motor responses to occasional targets</td>
</tr>
<tr>
<td>**Iowa Gambling Task (IGT)**414</td>
<td>Measures decision making, emotional processing and working memory</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Boston Naming Test: 30-item version</strong>415</td>
<td>Confrontational naming task, measures word retrieval for nouns</td>
</tr>
<tr>
<td>**Psycholinguistic Assessment of Language Processing Abilities (PALPA)**416</td>
<td>Word Reading (Regularity) Measures reading ability</td>
</tr>
<tr>
<td><strong>Memory (Visuospatial abilities)</strong></td>
<td></td>
</tr>
<tr>
<td>**Rey Auditory Verbal Learning Test (RAVLT)**417</td>
<td>Assesses encoding and acquisition of new information, attention, susceptibility to proactive and procreative interference, spontaneous recall and recognition of the information</td>
</tr>
<tr>
<td>**Logical Memory (WMS-III)**418</td>
<td>Assesses encoding and acquisition of new information, attention, spontaneous recall and recognition of the information</td>
</tr>
<tr>
<td><strong>Rey Complex Figure Test</strong>419</td>
<td>Evaluates visuospatial and constructive abilities and visual memory abilities</td>
</tr>
</tbody>
</table>
## Social Cognition

### Reading the Mind in the Eyes
- Measures the ability to perceive, recognize and name facial affect.

### Behaviour

<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beaumont Behavioural Inventory (BBI)</strong></td>
<td>Assesses behavioural changes, reported by both the participant and person who knows them well.</td>
</tr>
<tr>
<td><strong>Frontal Systems Behavioural Scale (FrsBe)</strong></td>
<td>Assesses behavioural changes, reported by both the participant and person who knows them well.</td>
</tr>
</tbody>
</table>

### Mood

<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospitalisation Anxiety and Depression Scale 2nd Version (HADS-II)</strong></td>
<td>Evaluate mood disturbance, particularly anxiety and depression.</td>
</tr>
<tr>
<td><strong>General Health Questionnaire: 12-item version (GHQ-12)</strong></td>
<td>Screening device for identifying non-psychotic and minor psychiatric disorders.</td>
</tr>
</tbody>
</table>

### B: Neuropsychiatric Assessment

<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UK Biobank Thoughts and Feelings Questionnaire</strong></td>
<td><strong>Patients Health Questionnaire-9 (PHQ-9)</strong>&lt;sup&gt;425&lt;/sup&gt; Measure presence and severity of depression.</td>
</tr>
<tr>
<td></td>
<td><strong>Generalised Anxiety Disorder-7 (GAD-7)</strong>&lt;sup&gt;426&lt;/sup&gt; Measure of presence and severity anxiety.</td>
</tr>
<tr>
<td></td>
<td><strong>Psychosis Screening Questionnaire (PSQ)</strong>&lt;sup&gt;427&lt;/sup&gt; Measures presence and severity of positive symptoms of psychosis and schizophrenia.</td>
</tr>
<tr>
<td></td>
<td><strong>Alcohol Use Disorders Identification Test (AUDIT)</strong>&lt;sup&gt;428&lt;/sup&gt; Measures presence and severity of excess alcohol usage.</td>
</tr>
<tr>
<td><strong>Obsessive-Compulsive Inventory Revised (OCI-R)</strong>&lt;sup&gt;429&lt;/sup&gt;</td>
<td>Assesses for obsessions and compulsions in 18 tasks including washing, checking, ordering, obsessing, hoarding and neutralising.</td>
</tr>
<tr>
<td><strong>Barrett Impulsiveness Scale (BIS-11)</strong>&lt;sup&gt;430&lt;/sup&gt;</td>
<td>Measures the presence of impulsive behaviours and preferences in five domains; attention, cognitive stability, perseverence, self-control and cognitive complexity.</td>
</tr>
<tr>
<td><strong>Dimensional Apathy Scale (DAS)</strong>&lt;sup&gt;431&lt;/sup&gt;</td>
<td>Assesses apathy in three dimensions; executive, emotional and cognitive/behavioural initiation.</td>
</tr>
<tr>
<td><strong>Autism Spectrum Quotient (AQ)</strong>&lt;sup&gt;432&lt;/sup&gt;</td>
<td>Assesses five domains including attention switching, communication, social skills, attention to detail and imagination.</td>
</tr>
<tr>
<td><strong>Adult ADHD Self-Report Scale (ASRS)</strong>&lt;sup&gt;433&lt;/sup&gt;</td>
<td>Measures symptoms of ADD/ADHD.</td>
</tr>
<tr>
<td><strong>Community Assessment of Psychic Experiences (CAPE-P15)</strong>&lt;sup&gt;434&lt;/sup&gt;</td>
<td>Measure of positive symptoms of psychosis; persecutory ideation, bizarre experiences and perceptual abnormalities.</td>
</tr>
<tr>
<td><strong>The Ten Item Personality Inventory (TIPI)</strong>&lt;sup&gt;435&lt;/sup&gt;</td>
<td>Assessment of the five main personality traits; extroversion, openness, agreeableness, neuroticism and conscientiousness.</td>
</tr>
</tbody>
</table>
4.4. **Neuropsychological Assessment**

A comprehensive battery of neuropsychological assessments were performed. The tasks utilized are described in detail below.

4.4.1. **General Cognitive Screen**

All participants completed a brief ALS-specific general cognitive screening assessment. The tool used is described in detail below.

4.4.1.1. **Edinburgh Cognitive and Behavioural ALS Screen (ECAS)**

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS)\(^{393}\) is a brief cognitive and behavioural screening tool which takes approximately 15 minutes to administer and can be performed by all trained healthcare professionals. Use of this multidomain screening tool in ALS patients has been validated as a means of determining the presence and severity of deficits in multiple cognitive domains. The ECAS is divided into two sections, the ALS-Specific section and ALS Non-Specific section, the scores of which are both reported independently and combined to provide an overall total score for the test. The ECAS ALS-Specific section assesses cognitive domains most commonly affected in ALS including 1) Language (Naming, Comprehension, Spelling), 2) Verbal Fluency (Free and Restricted Verbal Fluency) and 3) Executive Functions (Reverse Digit Span, Alternation, Sentence Completion, Social Cognition). The ECAS ALS Non-Specific section assesses cognitive domains which are thought to be preserved in ALS including 1) Memory (Immediate Recall, Delayed Percent Retention, Recognition) and 2) Visuospatial abilities (Dot Counting, Cube Counting, Number Location). The ECAS test accommodates for both limb and bulbar motor disability in ALS patients as it can be administered in both written and verbal form. Its use has been validated against other screening tools including the Montreal Cognitive Assessment (MOCA) and the Frontal Assessment Battery (FAB)\(^{436}\) and against full neuropsychological battery assessments\(^{393, 408}\). The ECAS includes a brief carer interview to assess for behavioural changes consistent with FTD. This portion of the ECAS was not administered to ALS relatives in favour of alternative behavioural assessment measures outlined below.

Scoring: The verbal form of the ECAS was administered for all relatives and controls. ECAS tests were scored with respect to age and education-adjusted Irish normative data\(^{408}\). For example, cut off values for normality for the ECAS Total Score in those younger than 65 years were 96 (if years of education < 12) and 100 (if years of education ≥ 12) respectively. Scores of less than these values were deemed abnormal. Scores on
the ECAS test range from 0 to 136, with lower scores indicating greater degree of cognitive deficits.

4.4.2. Current/pre-morbid intellectual functioning

Both pre-morbid and current intellectual functioning were assessed using two tasks, detailed below.

4.4.2.1. Test of Premorbid Function UK (TOPF-UK)

The Test of Premorbid Function UK (TOPF-UK) is a brief word reading test used to determine an estimate of an individual’s premorbid intelligence. Reading and intelligence show strong correlation and the TOPF correlates strongly with the WASI-IV. As such skills are felt to be relatively resistant to damage compared to other skills, the TOPF pertains to assesses knowledge obtained prior to the onset of neurological disease or insult. Subjects are required to read aloud an increasingly difficult list of words with atypical spelling, with test discontinuation when five consecutive errors are made.

Scoring: The total number of correct responses given is recorded. Age, gender and education-adjusted normative data is used to determine predicted IQ scores including full scale IQ (FSIQ), verbal comprehension index (VCI), perceptual reasoning index (PRI), working memory index (WMI) and processing speed index (PSI).

4.4.2.2. Wechsler Abbreviated Scale of Intelligence

The Wechsler Abbreviated Scale of Intelligence - Second Edition (WASI-II) provides a brief and reliable measure of intellectual functioning. It comprises four sub-tests (Similarities, Block Design, Vocabulary and Matrix Reasoning) of which the latter two may be used independently to estimate an individual’s full scale intelligence quotient (IQ). This briefer method of IQ estimation is often used for research purposes for pre-experimental matching of cognitive ability. This IQ estimate was used in our study to minimise the time demands placed on the subject by the neuropsychological assessment. The vocabulary sub-task measures the subject’s word knowledge, knowledge fund and language development. The matrix reasoning sub-task challenges subjects on four types of items: pattern completion, classification, analogy and serial reasoning.

Vocabulary: The subject is asked to provide definitions for words that the researcher reads aloud and the researcher records the subject’s responses verbatim. The subject is awarded 2 points for responses which demonstrate good understanding of the word.
e.g., synonym, definitive primary feature etc. 1 point is awarded for responses which are
correct but lack content, with 0 points awarded for responses which show no clear
understanding of the word. If the subject provides multiple responses for an item, they
are scored for their best response. Sample responses are available to the researcher to
guide how points are awarded for responses. The assessment is discontinued after 3
consecutive scores of 0. The examiner may query any response the subject provides
that is unclear or too vague to be scored. The first two items in the subtask are scored
either 2 or 0 only.

Matrix reasoning: The researcher presents the subject with an incomplete matrix or
series and multiple response options. The subject is then required to choose the correct
response option necessary to complete the matrix or series. Correct responses score 1
point and incorrect responses score 0. The assessment is discontinued after 3
consecutive scores of 0. Prior to commencing the sub-task, the subject is provided with
two sample items where they may receive corrective feedback and assistance from the
researcher. These sample items are not scored.

Scoring: Raw scores for vocabulary and matrix reason were converted to age-adjusted
T-scores and the sum of the T Scores convert to full scale IQ (FSIQ-2) in accordance
with the WASI-II user manual410.

4.4.3. Executive functioning

Five tasks were selected to assess executive function, chosen to provide a
comprehensive assessment of a heterogenous cognitive domain while minimising the
total time required to complete all tasks.

4.4.3.1. Verbal Fluency

Phonemic verbal fluency difficulties are the most consistent deficit observed in patients
with ALS and are largely thought to result from impairments in executive function, rather
than represent primary linguistic difficulties440. Executive processes required for verbal
fluency tasks include initiation of and switching between effective retrieval strategies and
inhibition of and monitoring for errors441. The restricted paradigm places greater
demands on executive function compared to the unrestricted paradigm and may
therefore be more sensitive to detecting such deficits440.

In this study, both the FAS test442 and four-letter long restricted paradigm440 phonemic
paradigms were used. In the former, the subject was asked to name as many words as
they could think of beginning with a certain letter within one minute. Letters F, A and S were used for three separate trials. For the phonemic restricted verbal fluency task, subjects were asked to name as many four-letter long words beginning with the letter C as they could in one minute. Subjects were prohibited from using names of people, places or numbers or the use of identical words with different endings (e.g., do and doing). Subjects were informed of all rules before commencing the task. The examiner recorded legibly on a sheet of paper all the words generated by the subject for each task and crossed out any incorrect words given by the subject. When all trials were completed, the subject was asked to read the list of words they generated for a particular letter (not including incorrect responses) as quickly as they could, while the examiner recorded the time it took them to do so. This time is an estimate of the time required by the subject to say the words without the requirement to retrieve them.

Scoring: The verbal fluency index (VFI) score was calculated for each trial (F, A, S, restricted C) and for total FAS test. The verbal fluency index score is a measure of the time taken to generate a new word in seconds, equivalent to the average thinking time per word. The verbal fluency index was calculated by first subtracting the time taken to read the list of generated words from the time given to complete the task. This value estimates the time it took the subject to think of the words. This value was then divided by the number of correct responses generated in the allotted time to give the verbal fluency index score (Formula A). For the total FAS Test verbal fluency index score, the total time to read all three lists of generated words was subtracted from the total time given to complete the task (60 seconds by 3 equals 180 seconds) and divided by the total combined number of correct responses generated for all three lists. Finally, the number of set-loss and repetition errors made were also recorded. The VFI score was used in all statistical analyses, with higher scores indicating worse performance.

$$VFI = \frac{\text{time given} - \text{time taken to read correct words generated}}{\text{number of correct words generated}}$$

Semantic (Categorical) Verbal Fluency

Similar to the procedure for phonemic verbal fluency, the subject was asked to name as many words as they could in one minute from a particular semantic category. In this study, that category was “animals”. Words generated by the subject were written down by the examiner and incorrect responses (set loss errors and repetitions) were crossed
out. When the trial was complete, the subject was timed as they read out the list of correct responses they had generated as quickly as possible. In addition to the executive and linguistic processes involved in phonemic verbal fluency, categorical verbal fluency tasks also involve temporal-lobe based semantic knowledge. The animal fluency task utilised in this study has been shown to correlate well with other semantic categories and also the FAS test.

Scoring: The verbal fluency index was calculated for categorical fluency using Formula A above. Rule violations (set-loss errors and repetitions) were also recorded.

4.4.3.2. Colour-Word Interference Test

The Stroop Colour Word Interference Test is one of the most commonly used executive function assessment tasks, both for its brevity and utility. It measures multiple dimensions of executive control including selective attention, inhibitory control, error monitoring, working memory and cognitive flexibility. Beyond this, it also provides useful measures of non-executive abilities such as processing speed.

Multiple versions of this test exist. The version chosen for this study was that from the Delis-Kaplan Executive Function System which consists of four separate tasks. The first two tasks are primers for the subject and assess key skills needed to complete the later tasks. In the first task, the subject is required to simply read a list of printed colours, while in the second task they are asked to read a list of words (comprising names of colours) aloud. The third inhibition task assesses the subject's ability to inhibit a salient response. The subject is asked to read through the list, naming the colour of the ink the word is printed in but not read out the word itself. The final task assesses both inhibition and switching abilities. The subject must alternate between naming the dissonant ink colour (as in the third task) and reading the word (when the word is in a box).

All tasks are to be completed as quickly as possible without making any mistakes. All tasks are timed by the examiner and the number of mistakes (corrected and uncorrected) are recorded. The subject is given two practice lines to read before the task begins to familiarise themselves with the task procedure which are untimed.

Scoring: Age-adjusted scaled scores are calculated from the time taken to complete each task and the total number of errors per task.

4.4.3.3. Digit Span

The Digit Span test is commonly used as a measure of attention, concentration, working memory, mental processing capacity as well as encoding and self-monitoring abilities. The Digit Span Backwards condition, in particular, challenges working memory and
inhibitory control\textsuperscript{444} while the Digit Span Sequential condition places extra demands on the semantic and phonological processing systems\textsuperscript{445}.

In total, three separate tasks are undertaken, involving forward, backward and sequential conditions. For the forward condition, the subject is requested to repeat series of digit strings called out by the examiner. For the backward condition, the subject is requested to repeat the series of digit strings, but in reverse order, for example if the examiner says 4-2-6, the subject is expected to reply 6-2-4. For the sequential condition, the subject is required to repeat the series of digits, but ordered in ascending order of magnitude, for example if the examiner says 4-2-6, the subject is expected to reply 2-4-6. For all tasks, the length of the digit string increases progressively, in pairs, to a maximum string length of nine digits. Tasks are discontinued once the subject gives two incorrect responses of the same digit length span.

Scoring: For each task, one point is awarded for each correctly repeated sequence. The total number of correct responses and the longest digit sequence span achieved was recorded for each task. The raw total score for each condition was converted to a Z-score which was used in all subsequent statistical analysis.

\textbf{4.4.3.4. Sustained Attention to Response Task}

The Sustained Attention to Response Task (SART)\textsuperscript{413} is a computer-based go/no-go task which is designed to measure one’s ability to inhibit responses to infrequently presented stimuli in the context of an established pre-potent motor response. As such, this tool provides a measure of an individual’s sustained attention, working memory and impulse/inhibitory control capacities. Recent work suggests that this task in combination with EEG may be a useful means to explore and quantify cognitive and behavioural decline in ALS\textsuperscript{446}, as the SART tool has greater sensitivity as a measure of sustained attention than other continuous performance tasks\textsuperscript{413}.

In this task, the subject is presented with the stimuli, consisting of a series of digits alternating with a mask (circle with a cross inside). Digits are displayed for 250ms, while the mask is displayed for 900ms. All stimuli are presented centrally on the computer screen, in white on a black background, in one of five randomly assigned font sizes (48, 72, 94, 100, and 120 point). The subject is required to respond (i.e., press the keypad) as fast as they can every time digits 0-2 or 4-9 are presented (go trial). Every time the digit 3 is presented, the subject is not to respond (no-go trial). Reaction times of all responses are recorded (response time). A training block with feedback on all responses (3 minutes) is followed by the assessment block (5 minutes). 7\% (18/260) trials per block are no-go trials.
Scoring: The number of omission, commission and anticipation errors and the response times before accurate and inaccurate responses are recorded automatically. Age-adjusted Z scores were generated automatically for total number of errors in each category for all subjects.

4.4.3.5. **Iowa Gambling Task**

The Iowa Gambling Task (IGT)\(^{414}\) is a tool used to measure decision making, risk discernment, emotional processing and working memory. This novel card-based gambling task assesses real world decision making through its design, as rewards and punishments are invoked in the face of uncertain premises and outcomes. It was originally developed as a means to identify those who prioritise immediate prospects over the potential future consequences of their actions, as seen in patients with ventromedial prefrontal cortex damage. Since then, this task has been employed to measure attention, executive function and risky behaviours in various clinical populations\(^{447}\). In patients with ALS, a differential performance profile with impaired capacity to learn to eschew disadvantageous stimuli has been observed through the use of this task\(^{185}\).

In this study, a computer-based version of the gambling task is used. The subject is required to select a card from one of 4 decks (A, B, C and D). One card is selected at a time and the subject is free to switch between decks at any time and as often as they like. The subject is given a “loan” of $2000 to start with and asked to make a profit. With every card selected, there is a possibility that the subject may win or lose money. The subject is not aware in advance of what each card will yield. All decks provide regular money pay-outs (reward) and irregular and unpredictable money loss (punishment). Decks A and B yield higher rewards for cards selected, while cards selected from decks C and D have a lower pay-out. Yet, punishments are also higher for decks A and B, such that these decks are disadvantageous in the long-term, as they result in a net loss of $250 in every 10 cards. In contrast, decks C and D have lower set punishments and are advantageous decks in that they result in a net gain of $250 in every 10 cards. Optimal performance by the subject requires that they learn which decks are most advantageous and shift their strategy accordingly. The task concludes once the subject has played 100 card selections.

Scoring: The net sum of money won or lost by the subject is recorded automatically.
4.4.4. Language

Two tasks were used to evaluate the language abilities of participants. These tasks are described below.

4.4.4.1. Boston Naming Test

The Boston Naming Test is a brief and highly informative screening test used commonly to assess word retrieval and semantic knowledge for nouns. As such, it is particularly useful for detection of and providing a measure of the degree of impairment in people with dysnomia. Furthermore, qualitative assessment of the class of errors (e.g., semantic, phonemic) can provide guidance as to the nature of the underlying language disorder.

The Boston Naming Test is a confrontational naming task where subjects are asked to name an object after being presented with a simple drawing of the same object. For this study, the 30-item version of the test was used. Where a subject was unable to correctly name the item, a semantic cue was provided. A subsequent phonemic cue was given if the subject had persistent difficulties. The 30 items used for this assessment are presented in order of word frequency.

Scoring: The total number of correct spontaneous responses was recorded as well as the number of items correctly named after provision of semantic or phonemic cues. A Z-score was obtained from the total number of correct spontaneous responses and used in all subsequent statistical analysis.

4.4.4.2. Psycholinguistic Assessment of Language Processing Abilities (PALPA) Word Reading (Regularity)

The Psycholinguistic Assessment of Language Processing Abilities (PALPA) Word Reading (Regularity) task assesses reading ability in consideration of the regularity of words. This is a sub-component of the PALPA linguistic battery which also allows for assessment of auditory and visual lexical processing abilities, word spelling, homophone recognition, spoken and written word and sentence picture matching and comprehension of verbs and adjectives. The word reading subtask was chosen for inclusion as part of the neuropsychological assessment battery for this study due to emerging evidence that ALS patients may have difficulties accessing irregular word forms at an orthographic input level which can contribute to word production abnormalities. This task requires the subject to read aloud a list of words, comprising both regular and irregular words.

Scoring: The number of errors (regular and irregular) was recorded.
4.4.5. Memory and Visuospatial abilities

Deficits in memory and visuospatial abilities were screened for using three tasks which are detailed below.

4.4.5.1. Rey Auditory Verbal Learning Test

The Rey Auditory Verbal Learning Test (RAVLT)\textsuperscript{1} is a brief neuropsychological tool of the auditory-verbal domain, which widely used to assess cognitive functions including attention and short-term memory (specifically evaluated in trials I and VI) and verbal learning and memory.

In this task, the examiner reads out a list of items (List A) five times which is immediately recalled by the subject after each presentation (trials I-V). The examiner then reads out a list of different items (List B) which is immediately recalled by the subject after which the subject is required to recall the items from List A again (trial VI). After a delay of 20 mins (during which other tasks in the neuropsychological battery were administered), the subject is required to recall the items of list A (trial VII). Finally, in trial VIII, the subject is required to identify all words pertaining to List A from recognition list read out by the examiner.

Scoring: The number of correctly recalled items per each trial (I-VII) is recorded. For trial VIII, the number of correct responses were recorded. Correct responses include correctly recognising all items on List A and identifying that all other items suggested by the examiner were not on List A. The number of true and false positives (intrusions) is recorded. Errors such as intrusions may suggest the construction of false memories with repetition of these intrusions suggesting monitoring difficulties\textsuperscript{4}. In this study, the total combined raw scores for trials I-V (total learning), as well as raw scores for trial VII (delayed recall) and trial VIII (total recognition) were converted to age and sex adjusted Z scores. These scores were used for statistical analysis.

4.4.5.2. Logical Memory

The Logical Memory (LM) subset of the Wechsler Memory Test-III\textsuperscript{2} is a tool used to assess verbal episodic memory. It measures multiple dimensions of episodic memory including attention, the ability to acquire and encode new information, conceptual organization, schema formation, spontaneous recall and recognition. The narrative nature of the recall challenges higher-level cognitive functions. As a result, it has been shown to be sensitive for detecting early cognitive decline\textsuperscript{5}.

The Logical Memory task comprises of three conditions (immediate recall [LM I], delayed recall [LM II] and delayed recognition [LM Recognition]). The examiner reads two brief
vignettes aloud. After each vignette, the subject is required to immediately repeat the vignette including as much detail as possible (LM I). In LM II, the subject is required to recall both vignettes after a 20-minute delay (during which other tasks in the neuropsychological battery were administered). Finally, in LM Recognition, the subject is asked a series of “yes” or “no” questions about each vignette to test their recognition of key story elements.

Scoring: The number of correct story units recounted under immediate and delayed recall conditions are recorded. Furthermore, the number of correctly recognised story units was recorded. Total raw scores for LM I, LM II and LM Recognition were obtained by combining the scores recorded for both vignettes. These raw scores were converted to age-adjusted scaled scores which were used for all statistical analyses.

4.4.5.3. Rey-Osterrieth Complex Figure Test

The Rey-Osterrieth Complex Figure Test (RCFT) is a widely used tool for assessing both visuoconstructive abilities and non-verbal memory. Originally developed by Rey (1941) and standardised by Osterrieth (1944), this task also places demands on executive function and perceptual organisation processes.

In this study, the version of the test used was that from Meyers & Meyers (1995) which consists of four separate sub-tasks. The subject is provided with a picture of a complex geometric figure. In the first sub-task, the subject is asked to copy this picture (Copy trial). The second sub-task requires the subject to redraw the figure in the absence of the stimuli (Immediate Recall trial). After a delay of 20 minutes, the subject is required to redraw the figure from memory (Delayed Recall trial). Finally, the subject is required to identify all key elements pertaining to the original geometric figure from a recognition sheet provided by the examiner (Recognition trial).

Scoring: The first three trials (Copy, Immediate Recall and Delayed Recall) were scored according to a standardised scoring system in which the geometric figure is divided into 18 units and points awarded for placement and accuracy of the trial drawing elements. For the final trial (Recognition), the total number of correct responses were recorded (correct recognition of key elements from the figure and non-inclusion of non-elements). Total raw scores from each trial were converted to age-adjusted Z scores, which were used for all statistical analyses.
4.4.6. Social Cognition

4.4.6.1. Reading the Mind in the Eyes

Deficits in social cognition are increasingly recognised as part of the cognitive profile of ALS\textsuperscript{454}, with debate as to whether they develop in parallel to executive dysfunction or not\textsuperscript{455}. The Reading the Mind in the Eyes Test (RMET)\textsuperscript{420} assesses theory of mind (i.e. the ability to infer how others are thinking and feeling) and challenges both emotional perception and language abilities\textsuperscript{456}.

In this 36-item task, the subject is required to review black and white photographs of peoples’ eyes. For each photograph, the subject is asked to choose which of four words provided best reflects what they believe the person is feeling or thinking. If the subject is not familiar with one of the word options, the examiner will provide them with a definition and example this word’s usage in line with the standardised administration protocol.

Scoring: The subject is awarded one point for each correct response. Total raw score was converted to Z score and which was used for all statistical analyses.

4.4.7. Behaviour

4.4.7.1. Beaumont Behavioural Inventory

Behavioural changes associated with ALS can be confounded by motor dysfunction associated with the condition. The Beaumont Behavioural Inventory (BBI)\textsuperscript{421} is an ALS-specific tool which allows for a brief yet comprehensive assessment of behavioural change, while correcting for the potential confounding impact of motor disability. This 41-item proxy-report questionnaire assesses a wide range of behavioural changes associated with ALS including behaviours linked to apathy, disinhibition, social cognition deficits, dietary changes, perseverative, stereotyped or obsessive-compulsive behaviours, utilisation behaviour, echolalia and altered response to sensory stimuli. Six questions relating to cognitive deficits in executive and language domains and two questions relating to psychotic symptoms are also included. The questionnaire is completed by someone who knows the subject well. They are asked to rate behavioural changes in respect to two timelines: ‘in the last 3-5 years’ and ‘in the last 10 years’ which allows for assessment of whether behavioural changes noted are longstanding or not. The former timeline is reflective of the subject’s current behavioural status.

Scoring: All behaviours are graded on a scale of 0-3, with 0 implying no changes and 1, 2 and 3 reflecting mild, moderate and severe changes respectively. Total scores for each timeline were recorded separately. Cut-off scores of ≥7 indicates a significant but mild
behavioural change while a score of ≥23 reflects severe behavioural changes in line with those seen in patients with FTD.421

4.4.7.2. Frontal Systems Behaviour Scale

The Frontal Systems Behaviour Scale (FrSBe) is an assessment tool used to measure and grade the severity of behavioural changes associated with frontal lobe damage. The characteristics measured by this scale are at least partially independent of those attributable to cognitive impairment and correlate with clinical outcomes, such as functional status.457 The scale has proven utility in detecting behavioural changes correlating with regional frontal lobe atrophy in patients with ALS, independent of physical disability.458, 459 Parallel forms exist to allow input from multiple sources including the subject, a family member or close caregiver or a staff member caring for the subject in a professional setting. In this study, the FrSBE Self and Family Versions were used and with both versions sharing identical content.

The subject (Self Version) is required to answer 46 questions with respect to the subject’s behaviour ‘3-5 years ago’ and ‘at the present time’. This allows for assessment of both the subject’s current and baseline behavioural status. Each question is scored on a 5-point scale from 1-5, with 1 stating that this behaviour ‘almost never’ occurs and 5 that it ‘almost always’ transpires. The subject is also asked to recruit a family member or close friend to complete the questionnaire (Family Version) in the same manner. The person completing the FrSBE (Family Version) is also the person who completes the BBI (see above).

Scoring: The FrSBE is scored with respect to the total count for all 46 items and three subscale scores correlating with behavioural clusters associated with frontal lobe damage: apathy, disinhibition and dysexecutive function. Total and subscale raw scores were converted to age, sex and education adjusted T scores and used for all statistical analyses.

4.4.8. Mood

4.4.8.1. Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a brief, reliable and valid instrument commonly used to measure symptoms of anxiety and depression in various clinical and non-clinical cohorts. In patients with ALS, a modified version of this tool has been created to account for potential confounding due to physical disabilities. In this population, this scale has been used directly to measure mood disturbances among
patients and also to control for the impact of psychological distress on neuropsychological assessment performance.

In this study, the modified version by Gibbons et al 2011\textsuperscript{53} was used to allow for comparison between participants and their relatives with ALS. This self-report questionnaire provides insight into the subject’s current emotional state as the subject answers questions in relation to their emotional well-being in the previous week. In total, the subject is required to answer 12 questions, equally split between those relating to anxiety or depression. Each question is scored on a 4-point scale from 0-3, with 0 denoting no concerns and 3 representing severe symptoms.

Scoring: Scores are tabulated separately for each factor. Revised cut-offs are proposed by Gibbons et al\textsuperscript{423}. Scores of 17-20 suggest a ‘possible mood disorder’ while scores of 21 or more suggest a ‘probable mood disorder’.

4.4.8.2. General Health Questionnaire-12

The General Health Questionnaire-12 (GHQ-12) is a self-administered screening tool, widely used to identify minor psychiatric disorders or psychological distress in the general population\textsuperscript{424}. Its psychometric properties are validated against those of the full questionnaire (GHQ-60), yet its brevity allows also for increased utility in the research context. In ALS research, this tool has been used to measure psychological distress among both patients and their caregivers\textsuperscript{463}.

The subject is required to answer 12 questions; 6 positively phrased and 6 negatively phrased. Each question is scored on a 4-point scale from 0-3. Positively phrased questions are scored as 0 = more so than usual, 1 = same as usual, 2 = less than usual and 3 = much less than usual. Negatively phrased questions are scored as 0 = not at all, 1 = no more than usual, 2 = rather more than usual and 3 = much more than usual. The scale provides a measure of the subject’s current psychological state as the subject is asked to consider their responses in relation to the previous few weeks.

Scoring: All questions are scored using the Likert scale approach (0, 1, 2, 3). A summated score is produced. Scores > 15 suggest some ‘evidence of distress’ and scores > 20 suggest ‘severe problems and psychological distress’.

4.5. Neuropsychiatric Assessment

A comprehensive battery of neuropsychiatric assessments were performed. The tasks utilized are described in detail below.
The UK Biobank Thoughts and Feelings Questionnaire\textsuperscript{464} is a composite questionnaire, derived from existing and validated measures, which aims to detect clinical and subclinical levels of psychiatric disorders including depression, anxiety, psychosis/schizophrenia and self-harm in the general population (Table 4-2). This questionnaire was developed by UK Biobank Mental Health expert working group. The UK Biobank is a large, population-based cohort study which aims to extensively characterize the prevalence and determinants of common life-threatening and disabling conditions\textsuperscript{465}. The central component of the 'Thoughts and Feelings' questionnaire is the Composite International Diagnostic Interview (CIDI) which solicits information on lifetime depressive, anxiety and psychosis\textsuperscript{466, 467}. It is supplemented with established measures of depression (Patients Health Questionnaire – 9 [PHQ-9])\textsuperscript{425}, anxiety (Generalised Anxiety Disorder – 7 [GAD-7])\textsuperscript{426}, alcohol usage (Alcohol Use Disorders Identification Test [AUDIT])\textsuperscript{428} and post-traumatic stress disorder (Post-traumatic stress disorder Check List – civilian Short version [PCL-6])\textsuperscript{468}. Where no existing measures were available to assess domains, the expert working group devised or adapted questions to identify requisite features taking into account participant acceptability and comparability of the measure\textsuperscript{464}.

Scoring: Case, control and exposure definitions are detailed by Davis et al (2020)\textsuperscript{464}. These definitions were determined as per tool specific cut-off thresholds or by consensus criteria agreed by the working group. Exposure definitions are self-imposed binary measures (e.g., Have you ever taken cannabis? Yes/No). All case definitions arise from self-reported data and are therefore classified as 'likely' rather than confirmed psychiatric disorders.

<table>
<thead>
<tr>
<th>UK Biobank ‘Thoughts and Feelings’ Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Domain/question topic</strong></td>
</tr>
<tr>
<td>A. Screening questions</td>
</tr>
<tr>
<td>B. Current Depression</td>
</tr>
<tr>
<td>B. Lifetime Depression</td>
</tr>
<tr>
<td>B. Lifetime manic symptoms</td>
</tr>
</tbody>
</table>
C. Current anxiety disorder

- Generalised Anxiety Disorder Questionnaire (GAD-7)\(^{426}\)

C. Lifetime anxiety disorder

- CIDI-SF\(^{466}\), anxiety module, lifetime version

D. Addictions

- Devised by the study team

E. Alcohol Use

- Alcohol Use Disorders Identification Test (AUDIT)\(^{428}\)

E. Cannabis Use

- Devised by the study team

F. Unusual experiences

- CIDI, psychosis module, lifetime version, abridged\(^{467}\)

G. Adverse events in childhood

- Childhood Trauma Screener – 5 items (CTS-5)\(^{469}\)

G. Adverse events in adult life

- Devised by the study team, based on existing questions

G. Post-traumatic stress disorder

- Post-traumatic stress disorder Check List – civilian short version (PCL-6)\(^{468}\)

H. Self-harm and suicidal thoughts

- Devised by the study team

J. Subjective wellbeing

- Devised by the study team, based on existing questions

K. Free-text box

From Davis et al (2020).\(^{464}\)

### 4.5.1.1. Obsessive-Compulsive Inventory – Revised

The Obsessive–Compulsive Inventory-Revised (OCI-R)\(^{429}\) is a brief, yet comprehensive self-report measure used to identify the nature of and determine the severity of obsessive and compulsive symptoms. It can be used also as a screening measure to detect possible OCD in the general population. In this task, the subject is required to answer 18 questions. Each item is measured on a 5-point scale from 0-4 with higher scores indicating greater distress relating to the symptoms. The 18-items relate to 6 clusters of obsessive or compulsive symptoms including washing, checking, ordering, obsessing, hoarding and neutralizing, each of which generates its own sub-scale score.

Scoring: The total score ranges from 0-72, with each subscale ranging from 0-12. Total scores of 21 or more indicate the likely presence of OCD.

### 4.5.1.2. Barratt Impulsiveness Scale

The Barratt Impulsiveness Scale (BIS-11)\(^{430}\) is a self-report questionnaire widely used to measure impulsive behaviours. It consists of six first-order factors which can be paired to produce 3 second order factors: attentional impulsivity), motor and perseverence (second-order: motor impulsivity), self-control and cognitive complexity (second-order: non-planning impulsivity). While this scale has not been employed in a large scale in ALS research, it widely used to measure trait impulsivity in other neurodegenerative disorders where this may be a concern e.g.
Parkinson’s disease. In this task, the subject is required to answer 30 questions, scored on a 4-point scale from 1-4, with 1= rarely/never, 2=occasionally, 3=often and 4=almost always/always.

Scoring: 11 items are reverse scored (4,3,2,1). The sum of all 30 scores is calculated to determine the total BIS-11 score, with a maximum score of 120. Scores of 72 or higher designate high impulsiveness, scores between 52-71 are considered within normal limits whiles scores of 51 or less represent overly controlled individuals.

4.5.1.3. Dimensional Apathy Scale

The Dimensional Apathy Scale (DAS) is a multidimensional assessment tool used to detect and classify modes of apathy. It is a particularly useful measure where apathy may be confounded by motor disability as direct reference to motor behaviours are avoided in each item. This tool has been used to identify characteristic and distinct apathy profiles in patients with ALS and Parkinson’s disease with prominent initiation apathy in the former and notable executive apathy in the latter. Furthermore, in patients with ALS, this tool has been used to identify specific associations between apathy subtypes and executive and emotional recognition dysfunction.

In this task, the subject must answer 24 questions, comprising items from 3 subscale scores: Executive, Emotional and Initiation apathy. Each item is measured on a 4-point Likert scale, from 0-3, with higher total scores in each dimension indicating higher levels of apathy.

Scoring: Subscale scores may range between 0-24, with a maximum possible total DAS score of 72. Total scores of 39 or more suggest significant apathy, with abnormality cut-off scores for Executive, Emotional and Initiation apathy subscales of 14, 15 and 16 respectively.

4.5.1.4. Autism Spectrum Quotient

The Autism Spectrum Quotient (AQ) is a self-administered measure of social and non-social, cognitive and behavioural traits associated with autism. It has utility in differentiating individuals with autism spectrum disorders from neurotypical individuals across various age categories. Case reports have suggested higher pre-morbid AQ scores in patients with bv-FTD as compared to those Alzheimer’s disease. The AQ task requires the subject to answer 50 questions, using a 4-point Likert scale model, with respect to five different areas of functioning: attention switching, communication, social skills, attention to detail and imagination.

Scoring: The 50 questions are split evenly between those expected to produce an “agree” response and those expected to produce a “disagree” response in people with
Asperger syndrome or high-functioning autism. 1 point is awarded for “Definitely agree” or “slightly agree” responses on items 1, 2, 4, 5, 6, 7, 9, 12, 13, 16, 18, 19, 20, 21, 22, 23, 26, 33, 35, 39, 41, 42, 43, 45 and 46. 1 point is awarded for “Definitely disagree” or “slightly disagree” responses for items 3, 8, 10, 11, 14, 15, 17, 24, 25, 27, 28, 29, 30, 31, 32, 34, 36, 37, 38, 40, 44, 47, 48, 49 and 50.

The total AQ score may range between 0-50, with higher scores consistent with a higher level of autistic traits. The AQ total score is continuously distributed in both an ASD and general population allowing for comparison of the extent of autistic traits across populations. Furthermore, a cut-off score of 32 or above is suggestive of a possible diagnosis of autism.

4.5.1.5. Adult ADHD Self-Report Scale

The Adult ADHD Self-Report Scale (ASRS)-Screener is a brief, self-assessment tool used to measure symptoms of attention deficit (hyperactivity) disorder (ADD/ADHD) based on the DSM-IV criteria. It encompasses 6 of the most predictive items from the complete 18 item Adult ADHD Self-Report Scale (ASRS), demonstrates excellent concordance with clinical evaluations, and indeed outperforms the more extensive tool as a screening measure. In the ALS population, lower pre-morbid adult attention deficit scores have been reported, supportive of epidemiological reports of higher business success and education attainment among people who develop ALS. In this task, the subject is asked to rate the frequency of 6 symptoms (4 inattention and 2 hyperactivity items), on a 5-point Likert scale of ‘never’, ‘rarely’, ‘sometimes’, ‘often’ or ‘very often’. Scoring: Responses ‘often’ and ‘very often’ for all 6 items and response ‘sometimes’ for items 1-3 are awarded 1 point, with a maximum possible total score of 6. A score of 4 or more is indicative of symptoms consistent with adult ADHD. A continuous score is also calculated to determine symptom severity. The Likert scale is adapted where 0 points is awarded for ‘never’ continuing up to 4 points for ‘very often’. In this way, the summed score of all 6 questions is reported.

4.5.1.6. Community Assessment of Psychic Experiences-Positive Scale

Psychotic-like-experiences (PLEs) are subclinical delusional and perceptual disturbances, which while usually transitory, are associated with an increased risk of future overt mental health problems. The Community Assessment of Psychic Experiences-Positive Scale (CAPE-P15) is a brief, self-report screening tool which measures the frequency of and distress associated with PLEs. It has been used in clinical and non-clinical populations to detect individuals at risk of developing psychotic disorders. Such, mild persecutory delusional disturbance has been observed in
patients in ALS and FTD, in particular in cases associated with the C9orf72 repeat expansion\textsuperscript{479}.

In this task, the subject completes 15 questions concerning psychotic-like-experiences using a 4-point Likert scale, from 1 (‘never’) to 4 (‘nearly always’). If the subject reports a psychotic experience, they are asked how distressing the experience was on a 4-point Likert scale, from 1 (‘not distressed’) to 4 (‘very distressed’). Questions are answered with respect to the previous 3 months, thus giving a current measure of symptoms. The 15 questions reflect a three-factor structure: Persecutory ideation (5 items), Bizarre experiences (7 items) and Perceptual abnormalities (3 items)\textsuperscript{480}.

Scoring: Weighed scores for frequency and distress of symptoms are calculated by dividing each sum score by the number of items completed\textsuperscript{481}. This is to account for non-response to any items. Higher scores reflect higher frequency and greater associated distress with psychotic-like experiences. For both frequency and distress, cut-off values of 1.2 and 1.09 are recommended for identification of those at ultra-high risk of developing psychosis (sensitivity 97.5\%)\textsuperscript{481}.

4.5.1.7. Ten Item Personality Inventory

The Ten Item Personality Inventory (TIPI)\textsuperscript{435} is a very brief measure of the 5 main personality traits; Extroversion, Openness, Agreeableness, Neuroticism and Conscientiousness. People with ALS score highly on Agreeableness, Conscientiousness, and Extraversion using similar measures, with the suggestion that these personality traits may contribute to the perception that people with ALS are relatively amiable\textsuperscript{482}. The TIPI task, requires the subject to complete 10 questions, scored on a 7-point Likert scale, from 1 ‘disagree strongly’ to 7 ‘agree strongly’, with each personality category comprising 2 items.

Scoring: Items 2, 4, 6, 8 and 10 are reverse scored (7-1). For each personality trait, an average score is calculated from the scores of the two items ([score + (recoded)score]/2).

4.6. Ethical Considerations and Data Protection

Ethical approval for this project was granted by Beaumont Hospital Ethics Medical Research Committee (REC reference 15/40). A data protection impact assessment (DPIA) was completed as part of the ethics application and reviewed by the Data Protection Officer in Beaumont Hospital. Comparison data for ALS patients was obtained from separate studies, also granted full ethical approval by the Beaumont Hospital Ethics Medical Research Committee. These studies examine the neuropsychological function (REC reference 13/102) and genetic (REF reference 05/49) aspects of ALS.
Recruitment for the study occurs through the ALS Clinic at Beaumont Hospital where patients are informed of the study and provided with information sheets for dissemination within families. Contact details for the research group are contained in the information leaflet. Any relatives who express an interest in participating receive a phone call where the details of the project are explained and information leaflet is emailed or posted to them. Relatives who attend clinic appointments with patients are approached directly about the project and provided with an information leaflet. Controls are recruited through a previously established control database.

All potential participants received a follow up phone call or email a minimum of one week after the initial contact to allow sufficient time to consider the project. All questions relating to the study are answered to the interested party’s satisfaction. For those who are interested in participating, a home visit is arranged where the study is explained again in detail. Written informed consent is obtained from the participant and co-signed by the researcher prior to any study procedures being performed.

The information leaflet and consent form are written in plain English with clear concise wording, explaining the rationale, procedures, risks and benefits of the study. They explain that participants may withdraw from the study at any point and that they may request that their data is deleted. Participants are informed that any future care would not be impacted by such a decision.

Participation in this study is purely for research purposes and it is not intended to provide any immediate benefit to the research participants. In the same manner, no harm is expected to happen to any participant as a result of the study. Temporary discomfort, bruising and localised bleeding may occur during phlebotomy. Participants can refuse to part-take in this or any other aspect of the study at any point. The neuropsychological assessment may be tiring or cause anxiety. Assessments are undertaken in the participants’ home or environment of their choice in order to minimise any undue stress. Participants are also made aware that they may take rest breaks or discontinue the assessment at any point. Neuropsychological test results are pseudonymised. Researchers and participants are not provided with individual tests results. However, should any clinical concerns arise during testing, the principal investigator would be informed and appropriate referrals made.

Only the data essential to achieve the outlined research objectives is collected, after informed consent has been obtained. All data collected is pseudonymised at source with the key retained by the Research Manager. The paper files are kept in a locked room at the Academic Unit of Neurology at Trinity College Dublin. Electronic data is encrypted.
and then stored in a password-protect, encrypted format on a secure server in Trinity College Dublin. Data is accessible only by the principal investigator and named researchers working directly on the project for the purpose of data analysis. All individuals processing the data have received data protection training.

Raw data is retained for a period of five years after completion of the project, after which point it will be destroyed. In line with best scientific practices for the publication and dissemination of research, anonymised data will be made available as open access online. Consent is obtained from study participants specifically for this purpose during the consent process.

DNA samples are collected on all participants for the purpose of genetic analysis. All donors provide informed consent for DNA to be retained indefinitely, as part of the ALS Relative DNA Biobank, for future research projects as approved by the Beaumont Hospital Ethics (Medical Research) Committee. DNA samples are codified at source and stored at the Smurfit Institute of Genetics in Trinity College Dublin. The codes are stored in a password-protected, encrypted format on a desktop computer in a locked room in the Academic Unit of Neurology, Trinity College Dublin. No individual results from genomic analyses are provided to participants or any third party. The researchers who collect the DNA samples and perform neuropsychological testing do not have access to individual genetic test results. In the same manner, those performing the genomic analysis to not have access to any personal identifiers.

4.7. Statistical Analyses

4.7.1. Power Analysis and Sample Size Calculations

A priori sample size calculations were performed to determine the minimum sample size necessary to ensure adequate statistical power to detect meaningful effects between groups. Calculations were performed with respect to two effects: 1) The difference in cognitive, behavioural and neuropsychiatric function between ALS relatives and healthy controls, and 2) The difference in cognitive, behavioural and neuropsychiatric function between ‘Familial’ ALS relatives and ‘Sporadic’ ALS relatives. For all calculations, the p-value for significance was set at 0.05, and desired power was 0.8, and formulas employing standardised mean differences approach were used.

Cognitive deficits, particularly executive dysfunction, are common in people with ALS and schizophrenia. Studies of unaffected relatives of people with schizophrenia have
identified such deficits as potential cognitive endophenotypes relating to genetic load\textsuperscript{367}. This study aims to assess whether such changes also occur in relatives of people with ALS. However, as this is an emerging area of scientific research, the available literature with respect to direct cognitive profiling of ALS relatives is sparse. As such, measures of effect sizes were drawn from the relevant schizophrenia literature. Verbal fluency deficiencies are the most consistent and sensitive deficit observed in patients with ALS and patients with- and relatives of- those with schizophrenia\textsuperscript{367, 440}. For this reason, sample size calculations were performed using effect sizes measures calculated for this variable.

4.7.1.1. Sample size calculation: The difference in cognitive function between ALS relatives and healthy controls.

The largest meta-analysis of cognitive endophenotypes in unaffected adult relatives of people with schizophrenia, was used for obtaining the data required for this sample size calculation\textsuperscript{367}. This study assessed cognitive test scores for 58 studies comprising 2872 relatives and 2457 healthy controls. Effect size (Cohen’s d) for verbal fluency was derived from data from 7 separate studies, using the formula below, and then weighed by the inverse variance when pooled across studies to calculate an overall mean effect size.

\[
d = \frac{\text{Mean (relatives)} - \text{Mean (controls)}}{\text{Pooled Standard Deviation}}\]

The weighed mean effect size for verbal fluency (d=0.48) was used to calculate the minimum sample size required per group using the formula:

\[
n = \frac{2}{d^2} \times \text{cp, power}
\]

In this case:

\[
n = \frac{2}{(0.48)^2} \times 7.9 = 68.58
\]

Therefore, a minimum sample size of 69 subjects per group is necessary to have sufficient power to detect meaningful differences between relatives and controls.
4.7.1.2. Sample size calculation: The difference in cognitive function between ‘Familial’ ALS relatives and ‘Sporadic’ ALS relatives.

The data required for this sample size calculation was obtained from a large study assessing verbal fluency as an endophenotype in first-degree relatives of patients with familial versus sporadic schizophrenia. 105 familial schizophrenia parents performed worse on verbal fluency testing compared with 207 sporadic schizophrenia parents (d=0.39). Using this effect size and the sample size formula above, the minimum sample size needed per group was calculated:

\[ n = \frac{2}{(0.39)^2} \times 7.9 = 103.88 \]

Thus, a minimum sample size of 104 subjects per group is essential to have sufficient power to detect differences between relatives of familial versus sporadic ALS patients.

Therefore, it is necessary to ensure that a minimum of 210 relatives of ALS patients (comprising equal numbers of familial and sporadic ALS relatives) and an optimum of 100 healthy controls are recruited to ensure sufficient power for analyses of primary study outcomes.

4.7.2. Statistical methods

At a group level, several statistical techniques were employed to analyse the data as described in detail below. All tests were two-sided with statistical significance declared for p < 0.05. Corrections for multiple comparisons and p-value adjustments were applied when necessary. Statistical analysis was conducted using IBM SPSS statistics 27.

For descriptive statistics, categorical variables were presented as frequency (and percentage). Normally and non-normally distributed continuous variables were described with means (and standard deviation) and medians (and interquartile range), respectively. Where data ascertainment was incomplete for individual cases, missing data was excluded from individual analyses but the cases were retained in the dataset.

For categorical variables, Pearson’s Chi-squared ($\chi^2$) test for independence was used to compare variable distributions. Fisher’s exact Probability Test was used when the minimum expected cell frequency was ≤ 5 in a 2 x 2 contingency table. For all continuous variables, the Shapiro-Wilk test was used to test normality of distribution and Levene’s test for equality of variance was used to test the assumption of homoscedasticity. Independent-samples t-test was used to examine the probability of a difference in mean
scores across two normally-distributed groups. The probability of difference between two non-normally distributed groups was tested using the Mann Whitney U test.

For comparison of 2 or more continuous variables, the probability of a difference between normally distributed variables was tested with the one-way ANOVA. Welch’s F test was employed when equal variances were not assumed. The Kruskal-Wallis test was used for non-normally distributed variables or when sample sizes were <30. Post hoc comparisons were performed as planned a priori. Bonferroni adjustments were applied to correct for multiple pairwise comparisons.

The degree of correlation between two continuous variables was expressed using Pearson’s correlation coefficient “r”. The square of the correlation coefficient (r²) was used to describe the proportion of the variation in one variable which was accounted for by variation in the other variable. Spearman’s correlation coefficient (rₛ) was reported for ordinal variables and small sample sizes (n <30). Correlations range between −1.0 and +1.0, with plus values representing positive correlations and minus values indicating negative correlations. The strength of the correlation was reported in Cohen’s (1998) guidelines: small effect = 0.10, medium effect = 0.30, moderate effect = 0.50, and large effect = 0.70.

Linear regression analyses were used to examine the relationship between continuous variables by establishing the extent to which the dependent variable was predicted by the independent variable. Simple or multiple linear regression were used to examine the impact of one predictor or ≥ 2 predictors respectively. For all regression analyses, assumptions were checked beforehand. The assumption of normality was assessed by visual inspection of normal Predicted Probability (P-P) plot comparing the distribution of standardized residuals to a normal distribution. Shapiro-Wilk test value of greater than 0.05 was also used to confirm that the residuals were normally distributed. A scatterplot of predicted values against residuals was inspected to confirm homoscedasticity of the residuals. Standardised residuals of value ±3 were excluded as outliers as were high influential cases (Cook’s distance >1). For multiple linear regression, the potential for multicollinearity (two or more predictor variables are highly correlated) was assessed by calculating variance inflation factor (VIF) values. Any predictor variable with a VIF value of ≥ 2 was excluded from the model.

Patients were followed from their time of diagnosis until death or censor date. For survival analysis, Kaplan-Meier curves were constructed with equality of outcome between groups assessed using the log-rank test. Cox proportional hazard regression was used to control for the effect of covariates on survival.
5. Chapter 5: Results Part I: Lifetime Risk and Heritability of ALS in a Well Characterised Population

Published Work List

The work described in Chapter 5 has been published in the peer-reviewed journal JAMA Neurology as:

5.1. Introduction

Chapter 5 and Chapter 6 address Aim 1 “To what extent does population-specific genetic signatures impact on ALS risk and phenotype”. In this chapter, I examine what proportion of the variation in the risk of developing ALS is attributable to genetic factors (Heritability), within the genetically homogenous Irish population (Sub-Aim 1.2). This first aim is achieved through a population-based parent-offspring heritability study. This pedigree study design assesses differences in phenotypic presentation across parent-offspring trios which allows one to calculate narrow-sense heritability (h²), an indication of the extent to which an exhibited phenotype will be transmitted from parent to offspring. This may then inform genetic counselling practices. As discussed in Chapter 2, estimating heritability in ALS has been problematic. As it is a rare disease, it has proven difficult to recruit sufficient numbers of participants for twin studies. Furthermore, the only parent-offspring study used to estimate ALS heritability was limited by use of indirect reported prevalence data rather than direct ALS incidence data for the population studied.

Pedigree studies estimate heritability based on parent-offspring concordance with reference to a liability threshold for disease, determined from incident data. The threshold of liability (discussed in Chapter 2) provides a fixed point by which to compare different populations with different incidences, as demonstrated in Figure 5-1. This liability model assumes an underlying normally distributed susceptibility for the disease within a population, where an individual’s total risk reflects the combination of genetic and non-genetic factors acting to alter an individual’s chance of developing a disease. Populations at greater genetic risk (e.g., relatives of probands) will have a higher mean liability than the general population. This is ultimately reflected in the higher disease incidence in the former. Heritability may be estimated with respect to the differences in mean disease liability between two populations. Importantly, the general population sampled to determine incidence must be representative of the population from which individuals with ALS and their parents were drawn. To examine this, it is also necessary to include an assessment as to whether the annual incidence of ALS varied significantly with time. This has been possible as the Irish ALS Register has been in operation for over 25 years.
5.1.1. Aims

The purpose of this study has been (1) to assess for evidence of temporal change in annual ALS incidence, (2) to determine the current lifetime risk of developing ALS in a well characterized population, (3) to estimate the heritability of ALS in the same population, and (4) to determine whether heritability is modulated by sex.
5.2. Methods

5.2.1. Participant selection

All incident ALS cases, with a confirmed diagnosis of Definite, Probable, Possible, or Laboratory supported ALS according to the El Escorial criteria, recorded on Irish ALS register between 1995 – 2017 were included in the study for the purpose of assessing of ALS incidence and lifetime risk. Since 2008, detailed family aggregation studies have provided additional family history information for those registered with the Irish ALS Register. As such, in deriving estimates of heritability, only those diagnosed between 2008 – 2017 were included, to optimise quality of family history information gathered and minimise impact of potential biases associated with long running registers. Furthermore, if more than one member of the same family was diagnosed during this period, the most recently diagnosed member was included in heritability calculations. Individuals with non-Irish parental origin were excluded. Data was cross-referenced with data from the Irish ALS DNA Biobank and all cases screened for the pathogenic C9orf72 repeat expansion were identified. Sex-specific ALS concordance rates were calculated (as described below), including only those in whom C9orf72 status was established and used to determine heritability estimates for the C9orf72 negative population. Five previously identified patients with sporadic ALS who carried other putative ALS-causative gene mutations (TARDBP [1], FUS [2], SOD1 [1], SQSTM1 [1]) were excluded from analysis.

5.2.2. Determining the heritability of ALS

To estimate the heritability of ALS within a population (step 3), it is necessary first to determine the current lifetime risk of developing ALS within this population (step 2). Prior to this, one must determine the annual ALS incidence within this population (step 1) (Table 5-1).

5.2.2.1. Step 1. Annual ALS incidence and temporal trends

Annual age and sex-specific standardised ALS incidence rates were calculated using Irish population data averaged over 5 census years (1996, 2002, 2006, 2011, 2016) and considering 5-year age groups from 15 to 85 years or over. The US 2010 population was used for standardisation purposes as it is the most frequently used standard population in ALS. To estimate temporal trends in ALS incidence, a simple linear regression line was fitted using calendar year as independent variable, assessing both overall and sex-specific incidence rates. Model assumptions were assessed using 1) normal P-P plot
which showed no deviations and 2) residual scatterplot which demonstrated no systematic variation (Std. residual range -1.4 to 2.2).

5.2.2.2. Step 2. Lifetime risk of ALS

The current probability method provides the gold standard estimate of lifetime risk, as it considers competing mortality risks\textsuperscript{488}. Using this method, estimations of the number of ALS cases that would develop in specific birth cohorts were calculated on the basis of the person-years at risk, drawn from the Irish life table No.16 2010 – 2012 and the age and sex-specific ALS incidence rates, calculated above.

Potential birth cohorts were identified for ALS patients and their parents. The average year of birth for patients was 1946 (males 1947, females 1944) and the average life expectancy for those surviving past childhood for the patient’s cohort was 71 for males and 74 for females. The average year of birth for patients’ parents was 1923 (males 1920, females 1925) and the average life expectancy for those surviving childhood was 66 for males and 67 for females. Sex-specific lifetime risk estimates of developing ALS were calculated using these life expectancy estimates and a combined average calculated.

All parents of ALS patients, who had also received a diagnosis of ALS, were identified. Where possible, affected parents were examined at the Irish national ALS clinic in Beaumont Hospital. For affected parents identified posthumously, diagnosis was confirmed using death certificates. The sex of affected parents was identified and matched with the sex of their affected offspring. Concordance rates were calculated by dividing the number of sex-specific ALS concordant pairs by the total number of sex-specific pairs (daughter-mother, son-mother, daughter-father, son-father).

5.2.2.3. Step 3. Heritability of ALS

Point estimates of heritability ($h^2$) and their standard errors were derived using the formulas below proposed by Falconer\textsuperscript{350}.

\[ h^2 = 2b \]

and

\[ SE = 2 \sqrt{V_b} \]
where

\[ b = \left( \frac{x_{gr} - x_r}{a_g} \right) \]

\[ V_b = [b(a - x)]_g^2 W_g + \left( \frac{1}{a} \right)_g^2 + (W_g + W_r) \]

and

\[ W = \frac{(1 - q)}{a^2 A} \]

\( h^2 \): narrow sense heritability; \( b \): parent-offspring regression coefficient; \( x \): deviation of threshold from mean; \( a \): mean deviation of affected individuals from the population mean; \( V \): sampling variance of \( b \); \( q \): observed incidence; \( A \): number of affected individuals in the sample from which the incidence is calculated; \( g \): sex-specific lifetime risk in the general population; \( r \): sex-specific lifetime risk in relatives.

\( x \) and \( a \) are the values corresponding to \( q \), taken from the table in Appendix A, Falconer, (1965)\textsuperscript{350}. \( x_{gr} \) is evaluated from the incidence in the general population comparable with the relatives and \( a_g \) is evaluated from the incidence in the general population comparable with the proband.
The threshold of liability was set using lifetime risk as calculated, for both patients and their parent’s generations (Figure 5-2). The parent-offspring regression coefficient (b) is the difference in the sex-specific deviation of the mean liability of both parents and offspring from the threshold \((x_{gr} - x_r)\) as a proportion of the mean deviation of affected individuals from the population mean \((a_g)^{350}\). Subscripts denote the sex-specific lifetime risk in the general population \((g)\) and in relatives \((r)\). Narrow-sense heritability \((h^2)\) is equal to twice the estimate of the parent-offspring regression coefficient \((h^2 = 2b)\). Calculations were performed for each sex-specific parent-offspring pairing independently with mean heritability and standard error estimates calculated weighted by the reciprocal of the sampling variance for each sex-specific parent-offspring pairing\(^{344}\).
<table>
<thead>
<tr>
<th>Step 3</th>
<th>Description</th>
<th>Formula</th>
<th>Data required</th>
<th>Alternative approaches</th>
<th>Justification for approach chosen</th>
</tr>
</thead>
</table>
| Heritability (h²)³ | The proportion of the variance in liability that is attributable to additive genetic factors | Falconer’s method<br>

\[ b = \left( x_{gr} - x_r \right) / a_g \]

\[ h^2 = 2b \]

\( b \): parent-offspring regression coefficient \( x \): deviation of threshold from mean \( a \): mean deviation of affected individuals from the population mean \( g \): sex-specific lifetime risk in the general population \( r \): sex-specific lifetime risk in relatives | 1. Number of affected individuals in the sample 2. Total number of individuals in the sample 3. Lifetime risk 4. Sex-specific parent-offspring concordance rates | Twin studies  • Co-variance among twins may vary with time  • Difficult recruiting sufficient numbers of twin in rare diseases  SNP data  • Limited to additive genetic variance captured by common genetic variants genotyped on a SNP array | Pedigree studies  • Increased power  • Concordance unlikely to vary  • Assessment of variation in liability attributable to genetic factors not limited by current testing methods |
<p>| Liability model ³⁵⁰ | The liability model assumes an underlying normally distributed susceptibility for the disease within a population, where an individual’s liability reflects the combination of genetic and non-genetic factors acting to alter an individual’s risk of developing a disease. | Modelled as a standard normal distribution with mean 0 and variance 1. | N/A | Binary model  • An individual either has the disease or not based on pre-defined diagnostic criteria | The liability model allows for an estimation of the variance in liability within a population, which is required to estimate heritability using a pedigree approach. Requires no prior knowledge about underlying genetic risk factors. |</p>
<table>
<thead>
<tr>
<th>Threshold of liability&lt;sup&gt;350&lt;/sup&gt;</th>
<th>The point on the scale of liability above which all individuals are affected and below which all are unaffected.</th>
<th>The standard normal deviate exceeded by the sex-specific lifetime risk in the population under study.</th>
<th>1. Sex-specific lifetime risk</th>
<th>N/A</th>
<th>Prerequisite for calculating heritability of a binary trait using Falconer’s method&lt;sup&gt;350&lt;/sup&gt;.</th>
</tr>
</thead>
</table>
| Concordance /Co-variance | Concordant parent-offspring pairs consist of a proband with ALS whose parent also has ALS<sup>344</sup>. | \[
\text{no. of concordant pairs} = \frac{\text{Pairs: sex-specific parent offspring pairs}}{\text{total no. of pairs}}
\] | 1. Number of concordant parent-offspring pairs<sup>4</sup> | N/A | Prerequisite for calculating heritability of a binary trait using Falconer’s method<sup>350</sup>. |
| Step 2 | Lifetime risk | The likelihood that a person will develop a certain disease during his or her lifetime. | Current probability method<sup>448</sup> | 1. Age and sex-specific ALS incidence rates | 2. Age structure of the population | 3. Birth cohort specific life-expectancies | Cumulative risk | Doesn’t take other competing risks into account | Tendency to overestimate the probability of developing a disease | Current probability method |
| | | | \[
p = \frac{1}{l_0} \sum_{x=1}^{g} L_x T_x
\] | t<sub>x</sub>: incidence rate in the age group x | L<sub>x</sub>: number of years lived by the survivors of age x during the age interval starting at x | l<sub>x</sub>: size of population at the beginning of the first age interval under consideration | Data extracted from population-specific period life expectancy tables for specific birth cohorts | Average year of birth for patients and their parents Period life expectancy at various ages for | | Considers competing mortality risks |
| Birth cohort specific life-expectancies | The average number of years a cohort is expected to live, based on the year of birth, age and sex. | Data extracted from population-specific period life expectancy tables for specific birth cohorts | 1. | N/A | Prerequisite for calculating lifetime risk. |
| Step 1 | Incidence rate | The rate of new cases per population at risk, over a specified time period. | $= \frac{\text{no. of disease onsets}}{\text{Sum of person} - \text{time @ risk}}$ | 1. Number of ALS cases diagnosed annually, considering 5-year age groups 15 to 85 years +  
2. Size of population at risk (census data)  
3. Standardised to US 2010 population | N/A | Prerequisite for calculating lifetime risk. The general population sampled must be representative of the population from which individuals with ALS and their parents were drawn. To examine this, it is also necessary to determine whether the annual incidence of ALS varied significantly with time |

*a: narrow-sense heritability reflects the proportion of variance in liability attributable to additive genetic variance; b: sex-specific pairs (daughter-mother, son-mother, daughter-father, son-father)*
5.3. Results

5.3.1. Demographics and Clinical characteristics

Comparison of demographics for both the overall incidence cohort (1995 – 2017) and heritability sub-cohort (2008 – 2017) are presented in Table 5-2. The higher incidence of cognitive-onset disease observed in the heritability sub-cohort likely reflects recent improved recognition of this phenomenon. Of those with a parental history of ALS, 57.1% were female and 22.0% were diagnosed with cognitive-onset ALS. Individuals with a parental history of ALS had younger age of onset (mean age 57.9 years, 95% CI 55.3 – 60.6) than the overall heritability sub-cohort (p<0.001). For nine cases, where age of onset was available for both affected parent and offspring, the affected offspring were younger at time of onset (mean age 52.0 years [95% CI 48.8 – 55.3]) compared with their parents (mean age 69.6 years [95% CI 62.4 – 76.9]) (p=0.008), despite no difference in time from onset to diagnosis between the groups (p=0.41).

Table 5-2. Comparison of demographic and clinical characteristics between overall study cohort (1995-2017) and heritability sub-cohort (2008-2017)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male (no., %)</td>
<td>1207 (56.7)</td>
<td>626 (56.0)</td>
<td>0.71</td>
</tr>
<tr>
<td>Age at</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset, years (mean, SD)</td>
<td>63.9 (11.6)</td>
<td>64.9 (11.3)</td>
<td>0.025</td>
</tr>
<tr>
<td>Diagnosis, years (mean, SD)</td>
<td>65.3 (11.6)</td>
<td>66.3 (11.3)</td>
<td>0.42</td>
</tr>
<tr>
<td>Onset to diagnosis, months (mean, SD)</td>
<td>14.2 (15.8)</td>
<td>14.0 (14.2)</td>
<td>0.78</td>
</tr>
<tr>
<td>Site of onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar (no., %)</td>
<td>734 (36.4)</td>
<td>382 (35.0)</td>
<td>0.46</td>
</tr>
<tr>
<td>Spinal (no., %)</td>
<td>1194 (59.2)</td>
<td>626 (57.4)</td>
<td>0.33</td>
</tr>
<tr>
<td>Cognitive (no., %)</td>
<td>50 (2.5)</td>
<td>45 (4.1)</td>
<td>0.011</td>
</tr>
<tr>
<td>El Escorial criteria at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite (no., %)</td>
<td>1002 (56.9)</td>
<td>510 (61.9)</td>
<td>0.015</td>
</tr>
<tr>
<td>Probable (no., %)</td>
<td>510 (28.9)</td>
<td>181 (21.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
5.4. Incidence and lifetime risk of ALS

2128 patients were diagnosed with ALS between 1995 and 2017. The annual age and sex-standardized ALS incidence did not change with time (p=0.14). The overall mean incidence rate was 3.1 (95% CI 2.9 – 3.2) per 100,000 persons. The mean male and female-specific annual incidence rates were 1.8 (95% CI 1.7 – 1.9) and 1.3 (95% CI 1.2 – 1.4) per 100,000 persons, respectively.

The current lifetime risk of developing ALS, adjusted for other cause mortality was 2.9 and 2.3 per 1,000 men and women respectively, corresponding to 1 in 347 men and 1 in 436 women. The mortality-adjusted lifetime risk of developing ALS during the patient’s average life expectancy was 1.8 and 1.5 per 1,000 men and women respectively. The mortality-adjusted lifetime risk of developing ALS during the patients’ parent’s average life expectancy was 1.2 and 0.9 per 1,000 men and women. The combined mortality-adjusted lifetime risk over both patients and their parents’ lifespan was 1.5 and 1.2 per 1,000 men and women.

5.5. Risk in relatives

Between 2008 through 2017, 1123 incident ALS cases were identified. 92 individuals were excluded (non-Irish parental origin [86], familial ALS [6]). 1117 patients were included in final analysis. Complete family history was available for both parents of all patients (n=2234). 32 parents had a diagnosis of ALS. Total and sex-specific concordance rates are reported in Table 5-3. Concordance was highest in female-female parent-offspring pairs. Concordance was similar for unlike-sexed relatives. Overall, in a population devoid of known Mendelian-inherited genes, the total lifetime risk of developing ALS was 0.7% in any first-degree relative of those with ALS (Table 5-3).

Table 5-3. Parent-offspring ALS concordance rates by overall heritability cohort and C9orf72 negative sub-cohort

<table>
<thead>
<tr>
<th>Heritability cohort (n=1117)</th>
<th>C9orf72 negative cohort (n=605)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>Parent</td>
</tr>
<tr>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>
5.5.1. Impact of C9orf72 repeat expansion

C9orf72 status was available for 674 patients diagnosed between 2008 – 2017. 69 patients were C9orf72 positive. 20% (14/69) of all C9orf72 positive patients reported a parental history of ALS. 32 patients had a parental history of ALS. 23 of these patients had C9orf72 testing performed, of whom, 61% (14/23) were C9orf72 positive. No other definitive ALS-causing mutation has been identified in these patients. 44% (4/9) of patients whose fathers had ALS carried the repeat expansion. By contrast, 71% (10/14) of patients whose mothers had ALS carried the C9orf72 repeat expansion.

5.6. Heritability of ALS

The overall mean lifetime heritability of ALS for the study cohort was 52.3% (95% CI 42.9 – 61.7). Lifetime heritability estimates by sexed-pairings and genetic status are displayed in Figure 5-3. Unlike-sexed relative pairings, that are sex equivalent, provided the most similar heritability estimates (mother-son 38.2% [95% CI 26.5 – 49.9]; father-daughter 34.3% [95% CI 21.4 – 47.2]). If ALS is considered as a polygenic disease, heritability should be equal for all parent-offspring pairings. As such, weighing the unlike-sexed relatives heritability estimates by the reciprocal of their variance, provides us a mean heritability estimate of 36.4% (95% CI 24.2 – 48.9). By contrast, heritability was significantly higher in sex-specific pairings (combined mother-daughter and father-son pairings 59.3% [95% CI 51.2 – 67.5]) (p=0.002). Overall heritability estimates were highest in female-specific pairings (mother-daughter 66.2% [95% CI 58.5 – 73.9]).

In C9orf72 negative patients, the overall mean lifetime heritability of ALS was 37.1% (95% CI 20.0 – 54.2). Female-specific heritability was 49.2% (95% CI 33.9 – 64.5). Heritability estimates between mother-son pairings were non-significant, which may be partially attributable to the low number of concordant ALS pairs in this group.
Figure 5-3: Sex-specific heritability estimates by overall heritability cohort and C9orf72 negative sub-cohort

<table>
<thead>
<tr>
<th></th>
<th>Heritability overall population %, (95% CI)</th>
<th>Heritability C9orf72 negative population %, (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>52.3 (42.9 – 61.7)</td>
<td>37.1 (20.0 – 54.2)</td>
</tr>
<tr>
<td>Daughter-mother</td>
<td>66.2 (58.5 – 73.9)</td>
<td>49.2 (33.9 – 64.5)</td>
</tr>
<tr>
<td>Son-mother</td>
<td>38.2 (26.5 – 49.9)</td>
<td>15.2 (-9.5 – 40.0)</td>
</tr>
<tr>
<td>Daughter-father</td>
<td>34.3 (21.4 – 47.2)</td>
<td>35.5 (17.3 – 53.8)</td>
</tr>
<tr>
<td>Son-father</td>
<td>50.1 (42.0 – 59.4)</td>
<td>34.5 (19.3 – 49.6)</td>
</tr>
</tbody>
</table>

Mean heritability estimates for A. Patient cohort 2008 – 2017 with lifetime risk of developing ALS of 1.5 and 1.2 per 1,000 males and females respectively; B. C9orf72 negative patient sub-cohort 2008 – 2017 with lifetime risk of developing ALS of 1.5 and 1.2 per 1,000 males and females respectively. C. Exact heritability estimates per study cohort and offspring-parent relationship.

5.7. Discussion

This is the largest population-based pedigree study conducted to date assessing ALS heritability. The mean annual age and sex-standardised ALS incidence and current lifetime risk of developing ALS observed were similar to those observed by other methods. The population-based heritability estimates of between 43 – 62% are similar to those calculated by Wingo et al344 (40 – 60%) using a US clinic-based population. However, in this study, ALS heritability was assessed for the first time in a population in whom known genetic mutations have been excluded. The heritability estimates
calculated were not significantly different in C9orf72 negative patients when compared with the overall heritability study cohort. Indeed, while the lifetime risk of developing ALS in first-degree relatives of individuals with ALS whose genetic status is unknown is 1.4%, first-degree relatives of individuals with ALS who are not known to carry any ALS-associated genetic mutations remained at increased risk of developing ALS (lifetime risk 0.7%) compared with the general population (lifetime risk 0.26%).

61% (14/23) of concordant ALS pairs, in whom DNA was available, carried the C9orf72 repeat expansion. By contrast only 20% of C9orf72 positive patients reported a parental history of ALS, suggesting that genetic anticipation or pleiotropic effects may have masked the clinical phenotype in their parents. The role of genetic pleiotropy with respect to the C9orf72 repeat expansion has been discussed previously in Chapter 1. In contrast, while it is interesting that, where data were available, the affected offspring of affected parents were on average 17 years younger at age of onset than their parents, overall clinical evidence supporting genetic anticipation in ALS is lacking. Anticipation is a well-known phenomenon in other trinucleotide repeat disorders including Huntington’s disease and spinocerebellar ataxias, where the repeat expansion length increases over successive generations associated with an earlier age of symptom onset and/or increasing severity of the phenotypic expression. Yet in ALS, this phenomenon among C9orf72 repeat expansion carriers has been relatively understudied in part due to technical difficulties with performing such analyses. The most commonly used method for measuring the repeat expansion (repeat primed PCR) does not accurately measure the exact expansion length above a certain limit, the minimum pathogenic repeat expansion length is undetermined and expansion lengths in peripheral blood samples may not correlate with the expansion length in neural tissues. Nonetheless, some clinical evidence of anticipation with respect to age of onset in C9orf72 families has been reported, but these studies are limited by small numbers of participants and a lack of sufficient genetic data correlating with clinical findings.

In this study, across all estimates calculated, mother-daughter pairings had the highest heritability, strongly suggesting a sex-mediated liability in this cohort. How this relates to ALS incidence can be reviewed in relation to the above figures, where the liability among women is represented in the top graph and among men in the bottom graph. As discussed in Chapter 2, the incidence of ALS is lower among women than men, as reflected in the lower mean liability in the female population. As such, affected females (who exceed the threshold of liability) deviate more from the mean of their sex than affected males do. If this liability is considered to some extent to result from genetic
factors, affected females are as such more likely to transmit this increased risk to their offspring. This is reflected in the higher heritability estimates observed in female-specific pairings than for unlike-sexed pairings. While female sex hormones have been suggested to modulate ALS risk$^{494,495}$ it is not possible to determine how these factors may impact on sex-specific heritability differences.

Parental sex has been observed to play a role on the stability of other diseases associated with nucleotide expansions. For example, in Huntington’s disease, an increase in the CAG trinucleotide expansion length occurs almost exclusively through paternal transmission. By contrast, in myotonic dystrophy 1, CTG trinucleotide expansions are more likely to be transmitted maternally. The observation of higher ALS heritability in females may be in part be driven by C9orf72 repeat expansion as 71% of patients whose mothers had ALS carried the repeat expansion. Indeed, the clinical phenotype and survival probability of C9orf72 expansions carriers have previously been shown to be influenced by sex$^{143}$, suggesting a complex interaction between sex, disease phenotype, and the repeat expansion. Nonetheless, female-specific heritability remained elevated also in C9orf72 negative populations, which suggests the possibility of additional uncharacterized repeat expansions associated with ALS. Further work is warranted to investigate this hypothesis.

5.7.1. Limitations

This study has several limitations. While no temporal change in ALS incidence in Ireland over the most recent 23 years was observed, it was not possible to determine whether incidence has changed over the entire period that patients and their parents were alive. Nonetheless, as ALS is a late-onset disease, the period assessed was that of most interest for these cohorts. Moreover, while the current probable lifetime risk describes the current risk of developing ALS for individuals of a certain age, it does not directly reflect the risk of either patients or parents at the time of their birth, nor is it predictive of future risk. Secondly, the impact of environmental variables was not directly assessed during this study. Of proposed environmental risk factors associated with ALS$^{133,139}$ other than smoking$^{496}$, no clear reported change in these variables has occurred over time. While it is possible that a changing environmental background over the last century may have impacted on heritability estimates, it seems unlikely that this would be the case given the stability of ALS incidence within the population. Finally, while I have accounted for all known genes of large effect in the Irish population, it is not possible to control for unknown genes of large effect. As discussed in Chapter 2, these mutations are more likely to arise in recent family lineage, with population variants more likely to act as
genetic modifiers\textsuperscript{254}. Nonetheless, the heritability estimates generated by this study support the ongoing search into the underlying genetic factors contributing to ALS risk and phenotypic expression to advance our understanding of the pathogenic mechanisms driving the disease.

5.7.2. Conclusions

In this chapter, I addressed the question to what extent do genetic factors impact on the variation in ALS risk, within a well characterised population. This is the largest population-based pedigree study assessing ALS heritability to date and the first study to assess heritability in the absence of known gene mutations of large effect. I have shown that approximately half the variation in ALS risk within a population is accounted for by genetic factors. Even excluding all known ALS genes of large effect, nearly 40% of the variation in risk is attributable to genetic factors, with relatives of “non-genetic” ALS probands remaining at increased risk of developing ALS compared with the general population, although this risk is small. Interestingly, I have shown for the first time that the heritability of ALS is highest among women suggesting that the disorder may be more likely to be transmitted maternally. This has previously been seen with other trinucleotide expansion disorders and further work will be required to assess whether other uncharacterized repeat expansions may also be associated with ALS.
6. Chapter 6: Results Part II: A comparison of the Clinical and Genetic features of ALS across Cuban, Uruguayan and Irish clinic-based populations

Published Work List

The work described in Chapter 6 has been published in the peer-reviewed journal Journal of Neurology, Neurosurgery and Psychiatry as:

6.1. Introduction

This chapter further explores the importance of population-specific ALS genetic signatures, by examining the extent of variation in ALS phenotypes across different populations, and assessing whether or not this can be explained by known ALS-associated variants (Sub-Aim 1.2) This is achieved through a clinic-based population study comparing demographic, clinical and family history data across two Latin-American (Cuba and Uruguay) and one European (Ireland) tertiary referral centres. Genomic analysis was conducted on samples from Cuban and Irish clinic-based populations.

As discussed in Chapter 2, the likelihood that population-specific genetics signatures drive population-specific disease patterns is supported in part by the wide geographic variance in the incidence and prevalence of ALS\(^{227, 497}\). Evidence of increased genetic susceptibility has been noted in isolated populations (e.g., Faroe Islands, Sardinia, Kii Peninsula in Japan and Guam)\(^{225, 227, 284, 333, 498}\), with relative isolates (such as Ireland) demonstrating higher levels of relatedness among apparently sporadic ALS patients than among controls\(^{325}\). Conversely, it is theorized that as genetically admixed populations contain a wider spectrum of at-risk alleles, this will result in a relative attenuation of both disease risk and phenotype. Indeed, and notwithstanding the relative lack of epidemiological studies in truly admixed populations, there is evolving evidence to suggest that population admixture may be protective.

Two large population-based mortality studies in truly admixed populations have suggested differential risk within populations of different ancestral origin\(^{230, 231}\). Firstly, a large Cuban study showed lower ALS rates in those of mixed ancestral origin compared with those of Spanish origin\(^{230}\). The Cuban population is highly admixed, comprising Spanish and African and indigenous ancestral groups with approximately one-third of the population of mixed ethnicity (combined European and African ancestry)\(^{324}\). Subsequently, a large Chilean population-based mortality study found ALS rates comparable to the Cuban population, with higher mortality rates in geographic populations of predominantly European ancestry compared with the national average\(^{231}\). Yet, whether other demographic and phenotypic characteristics differ between admixed populations and those of Northern European extraction remains unknown.

Here, I report on the demographic and clinical characteristics of patients with ALS within two large clinic-based populations from the province of Havana and its environs in Cuba, and in Montevideo in Uruguay and compare these with Irish clinic-based population data
collected at the same time. I also compare for the first time the frequency of known ALS genes in clinic-based populations from Havana and within the Irish ALS population.

6.2. Methods

6.2.1. Study populations

The National Institute of Neurology, Havana, Cuba is a publicly-funded, university-affiliated hospital and tertiary referral system for all of Cuba for neurodegenerative conditions. This centre, alongside other tertiary referral centres, is supported by the secondary health system which encompasses neurological, rehabilitation and palliative services from the 52 provincial and municipal university-affiliated hospitals. Extrapolating from data obtained from a Cuban population-based mortality study and Cuban census data, the 115 ALS patients captured by this clinic likely reflects 20-30% of all Havana based ALS patients diagnosed during the study period. While extrapolating incidence data from mortality studies is not usually reliable, one review comparing incidence versus mortality data in ALS identified this study as one of three high quality studies assessing ALS mortality rates, which likely provide an accurate reflection of the population ALS incidence rate.

By contrast, more than half of the Uruguayan population live in its capital city, Montevideo. Here, the Institution of Neurology, Hospital Clinics serve as a national tertiary referral centre for neurodegenerative conditions covering 19 administrative regions. Extrapolating from data obtained from a Uruguay-based incidence study, the 220 ALS patients captured by this clinic likely reflects approximately 40% of all Uruguayans diagnosed with ALS and living in Montevideo during the study period. Finally, the Irish national ALS clinic in Beaumont Hospital, Dublin is a public clinic serving as a referral centre for the entire Irish population since its foundation in 1994. The majority of individuals diagnosed with ALS in Ireland attend this clinic. The overall population estimates for Cuba, Uruguay and the Republic of Ireland at the mid-point of this study were 11.2, 3.3 and 4.2 million respectively. This work was conducted as part of the Latin-American Epidemiological Network of ALS (LAENALS) consortium efforts, examining genetic and environmental risk factors in Latin America and Europe.

6.2.2. Data collection and analysis

All patients, who attended the specialist clinics and were diagnosed with Definite, Probable or Possible ALS as defined by El Escorial criteria, between 1996 and 2017, were recruited for the study. Those with PMA and PLS were excluded. All patients had
detailed histories and complete general and neurological examinations carried out by the attending neurologists. Demographic information collected included date of birth, date of onset, date of diagnosis, date of last follow up or date of death, self-reported sex, self-reported ethnicity and province of residency. Clinical information collected included site of onset, first clinical symptom and prescription of Riluzole. Extensive family pedigrees were detailed from all patients reporting a definite or probable family history of ALS as defined by Byrne criteria\textsuperscript{246}. In line with official Cuban guidance, Cuban patients in the study were stratified by ethnicity according to self-reported skin colour (“white”, “black” or “mulatto”). Data analysis was carried out for the following variables: age at onset, age at diagnosis, self-reported sex, self-reported ethnicity, family history, site of onset, Riluzole prescription, onset to diagnosis interval, survival from onset and survival from diagnosis. For survival analyses, censor date was set as 31\textsuperscript{st} December 2017. Informed written consent was obtained from all participants and the study was approved by the research and ethics committees of all participating institutes.

6.2.3. Genomic analysis

DNA extracted from venous leucocytes were collected from all participating Cuban patients and controls who were phenotypically normal at the time of sampling. Cuban controls included spouses of patients and volunteers recruited nationwide through primary care offices. DNA samples were collected from all patients on the Irish ALS register, who attended the Irish national ALS clinic in Beaumont Hospital\textsuperscript{145}. Screening for the presence of the pathogenic C9orf72 repeat expansion in all Cuban patients and 832 Irish patients was performed using repeat-primed PCR with amplified fragments measurement by capillary electrophoresis on an Applied Biosystems 3500 Series Genetic Analyzer and visualised using Gene Mapper v.4.0, as described previously\textsuperscript{141}. A cut-off value of 30 hexanucleotide repeats or above was used to categorise samples as positive for the repeat expansion\textsuperscript{141}.

For 126 Cuban cases, 111 Cuban controls, 404 Irish cases and 310 Irish controls, targeted 300bp single-end next-generation DNA sequencing was carried out on an Illumina MiSeq. The exons of 37 genes reliably linked to ALS or dementia (ALS2, ANG, C21orf2, CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERBB4, FIG4, FUS, GRN, hnRNPA1, LMNB1, MAPT, MATR3, NEFH, NEK1, OPTN, PFN1, PRPH, PSEN1, PSEN2, SARM1, SETX, SIGMAR1, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TBK1, UBQLN2, UNC13A, VAPB, VCP) were targeted. Additionally, 272 Irish patients and 136 Irish controls underwent Illumina PCR free whole genome sequencing. Known ALS variants were defined as those which had an alternate allele frequency
below 1% in controls or 5% in reference population datasets and which were present in the ALS online genetics database (ALSoD)\(^4\). Genomic analysis for all samples was performed in house in the Smurfit Institute of Genetics, Trinity College Dublin.

### 6.3. Results: Demographics and clinical characteristics by population origin

The demographic and clinical characteristics of 115 Cuban and 220 Uruguayan ALS patients were analysed. Genetic data, available for all Cuban patients, were compared with that from 111 geographically matched phenotypically normal controls. In addition, the demographic and clinical characteristics of 1038 Irish clinic-based ALS patients were available for comparison. Genetic data were available for 832 of these. The demographic and clinical characteristics of the study populations are described in Table 6-1, below.

Allowing for differences in respect to cohort size, considerable variance in respect to age of onset and diagnosis was observed between populations (p<0.001). The Cuban population demonstrated the earliest ages of onset and diagnosis of 53.0 (95%CI 50.4, 55.6) and 54.4 (95%CI 51.9, 56.9) years respectively, with the mean age of onset and diagnosis occurring approximately 9 years later in the Irish population (age of onset 61.6 years [95% CI 60.9, 62.4]; age of diagnosis 63.3 years [95% CI 62.3, 63.7]). The age of onset and diagnosis in the Uruguayan population was 58.2 (95% CI 56.5, 60.0) and 59.5 (95% CI 57.8, 60.3) years respectively. Despite lower Riluzole prescription rates in the Cuban and Uruguayan populations (Riluzole prescription (%); Cuba 37.5, Ireland 83.1, Uruguay 25.1; p<0.001), no differences in survival durations were observed between populations (survival from diagnosis, months, median (interquartile range); Cuba 26.0 (13.0, 48.0), Ireland 21.0 (12.0, 41.0); Uruguay 23.5 (12.8, 42.9); p=0.72) (Figure 6-1). Mean length of follow up was Cuba 30 months (95% CI 24.3, 35.6), Ireland 27.1 months (95% 25.2, 29.0) and Uruguay 29.1 months (95% CI 24.7, 33.5). No differences in sex composition or site of disease onset between populations were observed.
Table 6-1. Comparison of demographic and clinical characteristics by population origin

<table>
<thead>
<tr>
<th></th>
<th>Cuba (n=115)</th>
<th>Ireland (n=1038)</th>
<th>Uruguay (n=220)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous variables (mean and 95% confidence interval)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>53.0 (50.4, 55.6)</td>
<td>61.6 (60.9, 62.4)</td>
<td>58.2 (56.5, 60.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>54.4 (51.9, 56.9)</td>
<td>63.0 (62.3, 63.7)</td>
<td>59.5 (57.8, 60.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Continuous variables (median and interquartile range)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset-diagnosis interval (months)</td>
<td>12.0 (7.0, 17.0)</td>
<td>11.0 (6.0, 19.0)</td>
<td>10.0 (5.1, 18.8)</td>
<td>0.79</td>
</tr>
<tr>
<td>Survival from onset (months), censored</td>
<td>39.0 (24.0, 70.0)</td>
<td>35.0 (23.0, 63.0)</td>
<td>36.9 (22.9, 71.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Survival from onset (months), uncensored (date death available)</td>
<td>30.0 (20.0, 55.0)</td>
<td>30.0 (20.0, 47.0)</td>
<td>32.0 (21.0, 47.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Survival from diagnosis (months), censored</td>
<td>26.0 (13.0, 48.0)</td>
<td>21.0 (12.0, 41.0)</td>
<td>23.5 (12.8, 42.9)</td>
<td>0.72</td>
</tr>
<tr>
<td>Survival from diagnosis (months), uncensored (date death available)</td>
<td>21.0 (10.0, 36.0)</td>
<td>17.0 (10.0, 29.0)</td>
<td>19.9 (10.8, 34.9)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Nominal variables (no., %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>63 (54.8)</td>
<td>611 (58.9)</td>
<td>136 (61.8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>70 (60.9)</td>
<td>657 (63.3)</td>
<td>146 (66.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>FALS present (yes)</td>
<td>18 (15.8)</td>
<td>122 (11.8)</td>
<td>11 (5.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Riluzole (prescribed)</td>
<td>42 (37.5)</td>
<td>863 (83.1)</td>
<td>55 (25.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 6-1. A). Kaplan-Meier plots of survival probabilities stratified by nationality for 1) onset to death and 2) diagnosis to death. B) Kaplan-Meier plot of survival probabilities for Cuban ALS population stratified by ethnicity for 1) onset to death and 2) diagnosis to death.
6.4. Impact of ethnicity on demographic and clinical characteristics

The demographic and clinical characteristics of the Cuban population, stratified by ethnicity are reported in Table 6-2. The majority of Cuban ALS patients were of self-reported “white” ethnicity (64.9%). No significant differences, as regards all assessed variables, were observed between groups.
### Table 6-2: Comparison of variables by self-reported ethnicity (Cuba)

<table>
<thead>
<tr>
<th></th>
<th>White (n=74)</th>
<th>Black (n=14)</th>
<th>Mulatto (n=26)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous variables (mean and 95% confidence interval)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>54 (51, 57)</td>
<td>54 (46, 63)</td>
<td>50 (44, 56)</td>
<td>0.48</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>55 (52, 58)</td>
<td>55 (48, 63)</td>
<td>51 (46, 57)</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>Continuous variables (median and interquartile range)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset-diagnosis interval (months)</td>
<td>12 (8, 18)</td>
<td>8 (6, 12)</td>
<td>11 (6, 21)</td>
<td>0.22</td>
</tr>
<tr>
<td>Survival from onset (months), censored</td>
<td>39 (24, 72)</td>
<td>32 (23, 54)</td>
<td>43 (32, 59)</td>
<td>0.79</td>
</tr>
<tr>
<td>Survival from onset (months), uncensored (date death available)</td>
<td>25 (20, 56)</td>
<td>24 (15,36)</td>
<td>39 (30, 55)</td>
<td>0.08</td>
</tr>
<tr>
<td>Survival from diagnosis (months), censored</td>
<td>25 (11, 50)</td>
<td>26 (12, 29)</td>
<td>28 (19, 40)</td>
<td>0.63</td>
</tr>
<tr>
<td>Survival from diagnosis (months), uncensored (date death available)</td>
<td>14 (9, 37)</td>
<td>13 (8, 29)</td>
<td>28 (18, 38)</td>
<td>0.74</td>
</tr>
<tr>
<td><strong>Nominal variables (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>38 (51)</td>
<td>7 (50)</td>
<td>17 (65)</td>
<td>0.44</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>40 (54)</td>
<td>9 (64)</td>
<td>20 (77)</td>
<td>0.12</td>
</tr>
<tr>
<td>FALS present (yes)</td>
<td>16 (22)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Riluzole (prescribed)</td>
<td>29 (41)</td>
<td>2 (14)</td>
<td>10 (39)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### 6.5. Genetic signatures: Known ALS associated mutations

5.2% (5/115) Cuban ALS patients were found to carry previously described disease associated variants (Table 6-3: ANG 1; CHCHD10 1; DCTN1 3). The pathogenic hexanucleotide repeat expansion in C9orf72 was identified by repeat-primed PCR in 2 patients, representing 1.7% (95% CI 0.6, 4.1) of all Cuban ALS patients (Table 6-3). Both carriers of the C9orf72 repeat expansion and one ANG mutation were detected in the “white” Cuban population. A DCTN1 (c.3746C>T(p.Thr1249Ile) variant was observed in the “black” Cuban population while a CHCHD10 (c.100C>T(p.Pro34Ser) mutation was observed in the “mulatto” population. 2.1% (2/96) individuals with sporadic ALS and 0/18 patients with familial ALS carried the C9orf72 repeat expansion. The proportion of Cuban patients with ALS was lower than observed in the Irish ALS population where 82/832 (9.9%, 95% CI 7.8, 12.0) patients were found to carry the repeat expansion (p=0.004). 5 additional Irish patients were found to carry known ALS-associated variants (TARDBP 1 FUS 2; SOD1 1; SQSTM1 1)487. No known SOD1, TARDBP or FUS mutations were found in the Cuban ALS population.
### 6.6. Discussion

This study is the first to describe the clinical and demographic characteristics of ALS phenotypes in Cuban and Uruguayan populations, with clinical implications not only for these groups, but also for their diaspora. The findings demonstrate a significantly lower age of onset and diagnosis in these two Latin American clinic-based cohorts compared to their Irish counterparts. Age of onset has been shown to vary between populations of different ancestries with a lower age of onset of ALS observed in some South American, Asian, and African populations which may be partially attributable to lower median ages across these populations (e.g. India and most African populations), compared with their Europe counterparts. However, examination of sex-specific life expectancies at birth and at 50 years of age published in the United Nations demographic yearbook at the mid-point of the study period reveals no notable differences between Cuban, Uruguayan and Irish populations (life expectancies at birth in years: (male) Cuba 75.1, Uruguay 72, Ireland 75.1; (female) Cuba 79, Uruguay 79.5, Ireland 80.3; life expectancies at 50 (years): (male) Cuba 28.5, Uruguay 26, Ireland 27.8; (female) Cuba 31.3, Uruguay 32.3, Ireland 31.9). It is generally considered that earlier onset of disease may reflect a major exposure to a risk factor, either genetic or environmental. This hypothesis aligns well with the multistep model of ALS, where recent work has shown a reduced number of steps in patients with ALS with genetic mutations. Indeed, evidence provided by a meta-analysis of genome-wide association samples, encompassing data from 13 European ancestry cohorts has identified six genomic regions associated with age at onset of ALS.

Younger age of onset is also considered a positive prognostic indicator in ALS. Yet, no significance difference in survival between the Cuban, Uruguayan and Irish cohorts was observed. For comparison purposes, Table 6-4. below details reported age of onset/diagnosis and survival for other Latin-American/ Hispanic populations. Current

---

### Table 6-3: Known ALS-associated variants in Cuban Population

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Patient Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG</td>
<td>c.250A&gt;G(p.Lys84Glu)</td>
<td>1/115</td>
</tr>
<tr>
<td>C9orf72</td>
<td>Repeat expansion</td>
<td>2/115</td>
</tr>
<tr>
<td>CHCHD10</td>
<td>c.100C&gt;T(p.Pro34Ser)</td>
<td>1/115</td>
</tr>
<tr>
<td>DCTN1</td>
<td>c.2353C&gt;T(p.Arg785Trp)</td>
<td>2/115</td>
</tr>
<tr>
<td>DCTN1</td>
<td>c.3746C&gt;T(p.Thr1249Ile)</td>
<td>1/115</td>
</tr>
</tbody>
</table>
evidence points to ALS as a multifactorial condition, resulting from a combination of genetic and environmental factors\textsuperscript{139}. The populations assessed in this study are very different in terms of environmental exposures, including care received following diagnosis. In Cuba and Uruguay, patients attend specialist clinics in Havana and Montevideo, respectively. While both clinics have access to Riluzole, NIV is not routinely available in Cuba, and is used as part of standard care in Ireland and Uruguay. Furthermore, Riluzole prescription rates varied significantly across sites. It is possible that this may have impacted on outcome, however it should also be noted that no difference in survival from onset was observed across the three populations. The data available did not permit a detailed exploration of risk in the context of environmental exposures and population genetics\textsuperscript{503}, and additional comparative studies are required.

Table 6-4: Age of onset and survival periods for Hispanic/Latino clinic-based populations

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>Age of onset (years; mean, 95%CI)</th>
<th>Survival interval (months; median, 95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monterrey, Mexico (2010)\textsuperscript{509}</td>
<td>61</td>
<td>47.5 (44.8, 50.2)</td>
<td>63.0 (49.5, 76.5)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47.0 (29.6, 64.4)\textsuperscript{b}</td>
</tr>
<tr>
<td>Havana, Cuba (2017)</td>
<td>115</td>
<td>53.0 (50.5, 55.6)</td>
<td>39.0 (30.6, 47.4)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.0 (17.0, 81.0)  \textsuperscript{c}</td>
<td>26.0 (21.6, 30.4)\textsuperscript{b}</td>
</tr>
<tr>
<td>Rio de Janeiro, Brazil (2007)\textsuperscript{504}</td>
<td>227</td>
<td>53.6 (41.5, 65.7)</td>
<td>49.0 (42.5, 55.5)\textsuperscript{a}</td>
</tr>
<tr>
<td>Montevideo, Uruguay (2017)</td>
<td>220</td>
<td>58.2 (56.5, 60.0)</td>
<td>36.9 (31.6, 42.3)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23.5 (19.5, 27.5)\textsuperscript{b}</td>
</tr>
<tr>
<td>Dublin, Ireland (2017)\textsuperscript{d}</td>
<td>1038</td>
<td>61.6 (60.9, 62.4)</td>
<td>35.0 (32.6, 37.4)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>63.0 (13.0, 91.0)  \textsuperscript{c}</td>
<td>21.0 (19.7, 22.3)\textsuperscript{b}</td>
</tr>
<tr>
<td>Barcelona, Spain (2001)\textsuperscript{510 e}</td>
<td>215</td>
<td>64.3 (29.0, 91.0)</td>
<td>30.8 (n/a)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.0 (n/a)\textsuperscript{b}</td>
</tr>
<tr>
<td>Santander, Spain (2013)\textsuperscript{511}</td>
<td>53</td>
<td>67.0 (50.0, 88.0)</td>
<td>22.0 (n/a)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

This study is the first to describe the genetic signature of ALS in a Caribbean population as explained by known ALS-associated variants. 5.2% of the Cuban clinic-based population had a known ALS-associated genetic variant (ANG 1; CHCHD10 1; DCTN1 3, C9orf72 2). The proportion of Cubans with ALS carrying the C9orf72 repeat expansion was significantly lower than their Irish counterparts (1.7% v 9.9%, p=0.004). Indeed, a lesser proportion of Cubans with sporadic ALS carried the C9orf72 repeat expansion (2.1%) compared to the carrier rate reported in a pooled analysis of European sporadic ALS cases (5.1%)\textsuperscript{249}. In keeping with the known variations in the frequency of C9orf72 repeat expansions across different populations, the proportion of Cubans with sporadic ALS carrying the C9orf72 repeat expansion was comparable to the rate seen in other Latin-American populations (Brazil 3.6%\textsuperscript{329}; Argentina 2%\textsuperscript{330}) and greater than that seen
in a pooled analysis of Asian sporadic ALS cases (0.3%)\textsuperscript{249}. No known SOD1, TARDBP or FUS mutations were found in the Cuban ALS cohort studied. In contrast, mutations in SOD1, TARDBP and FUS are among the most common genetically identifiable causes for ALS in European and Asian populations.

Given the relatively high proportion of familial ALS in the Cuban population, the paucity of genetically identifiable causes of ALS as regards known ALS-associated genetics variants is somewhat surprising. It is possible that the high familial rate may reflect the existence of as of yet unidentified rare ALS-associated variants of large effect within the Cuban population. Further studies of these Cuban familial ALS pedigrees are required to assess this hypothesis.

6.6.1. Limitations

This study was limited by data from clinic-based series rather than direct population-based measures. Those attending specialist clinics are often younger than true population-based samples and are more likely to have familial disease, and this could have biased the data. However, the majority of individuals diagnosed with ALS at the Havana and Montevideo clinics were from these regions or their surrounds. Moreover, the age at death within the cohort mirrored that of the population-based mortality study, and the proportion of patients within each ancestral population was also broadly reflective of that identified by the mortality study.

This study was also limited by the absence of genetic information available for the Uruguayan population. A more detailed genomic analysis of the Cuban population was limited by available material. Targeted sequencing was performing considering only known genes reliably linked to ALS or dementia, and it was not possible to comment on the existence of as-of-yet unknown genes of large effect or other potential ALS risk alleles within the Cuban population. Although the degree of genetic admixture could not be assessed using available samples, previous studies have shown that within Cuba and Uruguay, there is evidence of variance in the extent of genetic admixture by geographic regions. Within Havana and its surrounds, between 65-84% of the population are identifiable as of European ancestry through the use of genetic markers, with between 11-24% and between 5-11% identifiable as of African and Native American ancestry respectively\textsuperscript{324}. Furthermore, while the vast of the Montevideo population are of European ancestry, approximately 8% are of either African or Native American ancestry\textsuperscript{512}.

Exploratory analysis in the Cuban study cohort revealed no difference in clinical or demographic variables between ethnicities, although interpretation of this is limited by
the small numbers in each sub-cohort and by the absence of characterisation of respiratory and cognitive onset ALS in overall study cohort. Potential phenotypic differences could act as hidden co-variants between different population groups and are important factors to account for when assessing differences in mortality rates in admixed populations. Indeed, the EURALS consortium, in a study of six population-based registries over a two-year period, has previously demonstrated the existence of differences in site of onset by ethnicity across Europe. Larger scale population-based genome-phenotype correlation studies, such as these are required to fully assess the impact of genetic admixture in Latin-American populations. The Latin-American Epidemiological Network of ALS (LAENALS) study will perform this task in both the Cuban and Uruguayan populations, along with assessing the admixed Chilean population. This study will also aim to address limitations in characterisation of phenotype particularly the cognitive aspects of ALS by training all involved in appropriate standardised cognitive assessment techniques. Additional clinical information including disease progression and environmental exposures will be captured. Finally, direct genome phenotype correlations will be optimised through use of ancestral markers alongside self-reported ethnicity.

6.6.2. Conclusion

In this chapter, I have added to the literature regarding ALS epidemiology in understudied Latin American populations and reported for the first time on the genetic signature with respect to known ALS genes in the Cuban population. This study describes for the first time the clinical characteristics of ALS in Cuban and Uruguayan populations and has identified differences in age of onset/diagnosis between these populations and their Irish counterparts. Furthermore, I have demonstrated differences in the population genetics of ALS between the Cuban and Irish cohorts, with a significantly lower rate of the pathogenic C9orf72 repeat expansion in the former. This supports the hypothesis that population-specific genetic signatures may impact on the clinical characteristics of ALS across different geographical regions. Yet work in this area is complicated by heterogeneity in clinical care among other potential co-variants. Larger scale genome-phenotype correlation studies based on multicentre international collaboration are required to fully examine this hypothesis. Finally, in the Cuban population a high proportion of familial ALS was observed, not accounted for by known ALS gene mutations. This familial clustering of ALS again highlights the importance of recent family lineage, suggesting the existence of a local founder effect in an ALS gene that has not yet been identified. Detailed characterisation of these families supported by genome sequencing may prove very informative to our understanding of ALS pathogenesis.
7. Chapter 7: Results Part III: An Exploration of how we currently define ‘Familial ALS’ and its Evolution over Time

Published Work List

The work described in Chapter 7 has been published in the peer-reviewed journal Neurology Genetics as:

7.1. **Introduction**

Chapters 7, 8 and 9 address Project Aim 2 ‘To identify which ALS kindreds carry the greatest burden of shared genetic risk between ALS and other neuropsychiatric disorders’. I have assessed this aim using three sequential steps. Firstly, the impact of current familial ALS classification criteria is examined. Next, which neuropsychiatric disorders cluster in ALS kindreds are identified and finally, when this clustering exceeds chance alone is determined. In this chapter, the impact of current familial ALS classification criteria (Sub-Aim 2.1) and the impact of recent advancements in our understanding the various genetic and phenotypic manifestation that occur within ALS kindreds (Sub-Aim 2.2) was explored using trends in familial ALS incidence.

ALS is often loosely categorised into “Familial ALS” (FALS), in which other family members are reported to have had ALS, and “Sporadic ALS” (SALS), where there is no discernible family history. This is because familial clustering of a disease is generally agreed to imply a certain shared genetic liability, although other shared exposures may also contribute to disease risk. Yet, what constitutes significant clustering in ALS kindreds remains unclear. In the face of a lack of consensus on how best to define familial disease\(^{245}\), Byrne et al (2011)\(^{246}\) proposed classification criteria with various levels of stringency. Since these criteria were published, however, our understanding of what constitutes familial disease has expanded to encompass numerous neuropsychiatric disorders. Whether the presence of these neuropsychiatric phenotypes within relatives should be considered in the definition of familial disease remains to be determined.

In this study, I have used Byrne’s criteria for familial ALS to assess temporal trends in FALS incidence rates in an Irish population over a 23-year period as a means to explore the evolution of thinking around the definition of FALS. Furthermore, I have evaluated how, and if, the recent advances in our understanding of the various genetic and phenotypic representations of ALS has impacted on familial ALS incidence.

7.2. **Methods**

Temporal trends in Familial ALS incidence were assessed using data extracted from Irish ALS Register, Irish ALS DNA Biobank and Irish Familial ALS database. Data on all incident ALS cases diagnosed between 1994 to 2016 were interrogated. All cases reporting a history of suspected or confirmed ALS or FTD in at least one relative were collated. The DNA database was cross-referenced with the clinical database and the genetic status was determined in all cases for whom DNA was available. Cases with a diagnosis of Kennedy’s disease were excluded. Individuals who had a family history
suspicious for ALS (e.g., relative died from “muscle wasting disease”), in whom we could not confirm the diagnosis, were excluded. The Byrne criteria for Familial ALS were applied to all identified FALS cases.

Annual age-standardised incidence rates for FALS and FALS sub-classifications were calculated as a proportion of the total number of ALS cases diagnosed annually, using the pooled Irish ALS register population from 1994 to 2016 and considering the following age bands: \( \leq 39 \), 40-49, 50-59, 60-69, 70-79 and 80+. Where multiple members of the same kindred were identified, detailed pedigrees were constructed where possible. All newly diagnosed individuals from previously identified kindreds were identified. Crude incidence rates of newly diagnosed individuals from known families were calculated as a proportion of total number of ALS families presenting annually. Dates of diagnosis of FALS for all cases were obtained from the ALS Register and cross-referenced against medical records where applicable. All cases were grouped by whether they were recategorized from sporadic ALS to FALS or identified as FALS at time of diagnosis. Annual rates of recategorization were calculated by dividing the number of recategorized FALS individuals by the number of individuals diagnosed with ALS annually.

All probands with a confirmed family history of FTD, all-type dementia and/or schizophrenia/psychosis during the study period were identified from the Irish ALS Register. The crude incidence rates of probands with a confirmed family history of each variable were calculated by dividing by the number of patients diagnosed with ALS annually. Data were extracted from the Irish ALS DNA biobank to identify all probands carrying an established Mendelian-inherited ALS gene variant and the crude incidence rate of Mendelian-inherited ALS was calculated by dividing by the number of patients diagnosed with ALS annually. Patients for whom DNA was not available and without confirmed family history of ALS or FTD were categorised as non-familial cases.

Linear regression models were fitted to estimate the annual mean change in incidence rates with calendar year as predictor variable and dependent variables: total FALS, definite FALS, probable FALS, possible FALS, FALS from previously identified families, recategorized FALS, Mendelian-inherited ALS and probands with positive family history of FTD, all-type dementia and schizophrenia/psychosis respectively. To determine whether increasing rates of FALS were a function of higher rates of “possible FALS” diagnoses, we tested the hypothesis that the total FALS (b1) and combined “definite” and “probable FALS” (b2) beta coefficients were not statistically different from each other. A simple linear regression model with total FALS and combined “definite” and “probable FALS” as main effects with joint interaction term (b1*b2) was fitted via bias
corrected bootstrap (1,000 re-samples). SPSS Statistics Version 24 was used to identify and estimate the parameters of the linear models and to test for statistical significance.

7.3. Results

7.3.1. Demographics

2173 individuals, diagnosed with ALS between 1994-2016, were recorded on the Irish ALS register. Of these, 313 individuals had potential FALS based on a family history of suspected or confirmed ALS or FTD in at least one relative, or the presence of Mendelian-inherited ALS gene mutation in the proband (Figure 7-1 A). 35 individuals with a family history suspicious for ALS or FTD in whom it was not possible to confirm the relatives’ diagnoses and 9 individuals with proven Kennedy’s disease were excluded. 269 registered ALS patients comprising 197 unique families were included in the final analysis (Figure 7-1 B). 94 individuals carried a known ALS-causative gene mutation (C9orf72 (89), TARDBP (1), FUS (2), SOD1 (1), SQSTM1 (1)). 51 patients carrying the C9orf72 variant reported a family history of FTD. Secondary analysis of the Irish ALS register identified 392 patients with a confirmed relative with dementia (Reported as Alzheimer’s (131), FTD (51), unspecified (210)) and 57 patients with a confirmed family history of schizophrenia.
A) All ALS patients registered with Irish ALS Register from 1994 through 2016 who reported a history of suspected or confirmed ALS or FTD in at least one relative were identified. All patients with an established, highly penetrant ALS variants (C9orf72 89, TARDBP 1, FUS 2, SOD1 1, SQSTM1 1) were identified from the DNA database. (B). 35 patients with a family history suspicious for ALS or FTD in whom it was not possible to confirm the relatives’ diagnoses and 9 patients with Kennedy’s disease were excluded. 6 C9orf72 positive patients with a family history suspicious for ALS or FTD in whom it was not possible to confirm the relatives’ diagnoses were recategorized as “gene-positive-FALS”.

7.4. Incidence of FALS: Impact of current classification criteria

The overall mean annual crude FALS incidence rate was 11.1% (95% CI, 8.9, 13.3) for the study period, and the corresponding mean age-standardised FALS incidence rate was 11.1% (95% CI, 8.8, 13.4). However, the age-standardised overall FALS incidence rate increased steadily from 5.2% in 1994 to 19.1% in 2016, representing an increase of
0.7% (95% CI, 0.5, 0.9, p<0.001) per annum (Figure 7-2 A). Using our previously published criteria for “definite FALS”, the mean age-standardised incidence rate was 4.0% (95% CI 2.9, 5.0) for the entire study period, increasing by 0.2% (95% CI, 0.0, 0.3, p=0.007) annually between 1994 and 2016 (Figure 7-2 B). Similarly, the age-standardised incidence for “possible FALS” increased by 0.4% (95% CI 0.2, 0.5, p<0.001) annually, with an overall mean rate of 4.6% (95% CI, 3.1, 6.1) (Figure 7-2 D). For “probable FALS”, the age-standardised incidence rate was 3.1% (95% CI, 2.3, 3.9), but did not increase with time (p=0.32) (Figure 7-2 C). There was no difference between the total FALS (b=0.007) and combined “definite” and “probable FALS” (b=0.003) beta coefficients (p=0.67) (Figure 7-2 E).

A mean of 2.9% (95% CI 1.8, 4.1) of individuals diagnosed with ALS each year were from known FALS families, increasing by 0.3% annually (95% CI 0.2, 0.4, p<0.001). The relative contribution of newly diagnosed individuals from known families increased annually (p=0.001), accounting for 50% of all FALS diagnoses in 2016. To prevent an underestimation of effect size due to unrecognised FALS in sporadic ALS individuals (i.e., those in whom a second family member has not yet been affected), the final 3 years of data collection were excluded. For the remaining years, the overall mean rate of recategorization from sporadic to familial ALS was 3.0% (95% CI 2.6, 3.8) annually. This did not change with time (p=0.18).
Figure 7-2: Temporal trends on age-standardised Familial ALS incidence

Temporal trends in age-standardised FALS incidence for Total FALS (A), Definite FALS (B), Probable FALS (C) and Possible FALS (D). Temporal trends in age-standardised FALS incidence for Total FALS compared with Definite & Probable FALS (E)
7.5. Incidence of FALS: Impact of recently identified genetic and phenotypic representations of ALS

7.5.1. Mendelian-inherited ALS

From 1999 to 2016, the mean crude incidence rate for known Mendelian-inherited ALS was 4.5% (95% CI 2.8, 6.2) annually. A temporal increase of 0.4% (95% CI 0.2, 0.6) annually (p=0.02) was observed, driven by C9orf72-positive ALS patients, who accounted for 89 out of 94 known Mendelian-inherited forms. The other cases, all of which were sporadic, were associated with mutations in TARDBP (1), FUS (2), SQSTM1 (1) and a previously recognized rare SOD1 variant.

7.5.2. Impact of phenotype

From 1994 to 2016, a mean of 1.9% (95% CI 1.1, 2.9) of individuals diagnosed with ALS annually had a confirmed positive family history of FTD, increasing by 0.2% (95% CI 0.0, 0.4; p=0.001) per year. The mean crude incidence rates of probands with confirmed family history of any form of dementia or schizophrenia were 14.9% (95% CI 9.3, 20.5) and 2.2% (95% CI 1.4, 3.0) respectively. Both demonstrated annual increases of 1.8% (95% CI 1.4, 2.2; p<0.001) and 0.2% (95% CI 0.2, 0.2; p<0.001) respectively.

7.6. Discussion

The Irish ALS population-based register is flexibly designed to allow for both the categorisation of FALS and the application of different levels of stringency of the definition of FALS. The availability of this data register, combined with island status, low immigration rates and comparatively large family sizes makes Ireland an ideal place to study FALS. By applying the Byrne criteria for FALS to the Irish ALS population-based Register, the mean annual age-standardised incidence rate for any definition of FALS over a 23-year period was identified as 11.1%, consistent with the commonly quoted 10% FALS incidence figure reported in other populations of European ancestry. Application of these stringent criteria also identified an increasing trend in in FALS ascertainment, ranging from 5.2% in 1994 to 19.1% in 2016, with over 50% of newly diagnosed FALS cases in 2016 coming from second generations within known FALS families. This observation explains the increasing trend in “Definite FALS”, a term used to describe kindreds with a proband and at least two other relatives with ALS. Identification of all the high risk FALS families within the population ensures optimum ascertainment of all new cases from these kindreds.
In contrast, the stability of temporal trends in “Probable FALS” incidence and rate of recategorization from “sporadic ALS” to “familial ALS” align in that both reflect ALS kindreds with a total of two relatives with ALS (proband and one other). Such kindreds account for up to 50% of FALS cases in some series\textsuperscript{514}, are less likely to harbour known pathogenic mutations\textsuperscript{351} and may reflect a chance occurrence in larger kindred sizes\textsuperscript{246}. The stability of both categories above likely reflects the constancy of the overall age-adjusted incidence of ALS among the general population\textsuperscript{515}.

The increasing incidence in “Possible FALS” over the study period is explained by two factors. The mean incidence rate of known Mendelian-inherited “genetic ALS” increased annually. This figure is driven by \textit{C9orf72}-positive ALS, which accounts for the vast majority of known Mendelian-inherited ALS in Ireland. It is difficult to explain why this would increase with time, as DNA samples were collected since 1999 as part of the Irish ALS DNA biobank allowing for retrospective testing for newly identified variants. It may in part reflect the increasing ascertainment of cases from “Definite FALS” kindreds. As such, one may expect that this effect may plateau with time but an expansion of this study conducted at a later date would be required to confirm this. The characteristics of the \textit{C9orf72}-positive ALS patients without confirmed family history of ALS or FTD are described in more detail in Appendix 2. The findings are in keeping with previous work from our group suggesting that truly “sporadic” ALS associated with \textit{C9orf72} repeat expansions are rare, and that the vast majority of those carrying this variant have a family history of either neurodegenerative or neuropsychiatric disease\textsuperscript{264}.

The increasing “Possible FALS” incidence is also driven by an increased trend in incidence of probands with confirmed positive family histories of FTD. Exploratory analysis confirms this increasing trend is also seen for probands with family histories of unspecified dementia and schizophrenia reflecting enhanced recognition of the importance of extended phenotypes within ALS kindreds. This systematic difference in clinical phenotype between historical cases and those more recently enrolled reflects an “information creep” phenomenon, which is a common occurrence in long-standing registers\textsuperscript{486}. Yet, this finding also highlights a growing lack of clarity as to how best to define significant familial clustering within ALS kindreds, taking account of these expanded phenotypes.

This study has limitations. The presence of the \textit{C9orf72} repeat expansion was determined by repeat-primed PCR plus amplicon length analysis in blood samples. While confirmation of each repeat expansion length using Southern Blotting is recommended, and while there is acknowledged variation in amplicon length across the tissues, the
approach used in this study was validated using positive and negative controls confirmed using Southern Blot, and is consistent with that adopted in the setting of diagnostic screening.

7.6.1. Conclusion

The definition and utility of the concept of FALS remains a matter of debate. In this chapter, study data demonstrate that the estimated frequency of FALS within a population can be biased by both ascertainment methods, the level of stringency applied to the definition, and the inclusion or exclusion of extended phenotypes and endophenotypes that are biologically associated with ALS. Applying the current stringent familial ALS classification criteria to longitudinal population-based register data indicates that, in Ireland, at least 20% of ALS is familial. However, while the importance of extended phenotypes within ALS kindreds is increasing recognised, there remains a lack of clarity as to how best to define significant familial clustering of these disorders. Nonetheless, there is increasing evidence to suggest that a wider diagnostic categorization of familial ALS, to include FTD and neuropsychiatric conditions, is warranted.
8. Chapter 8: Results Part IV: Identification of Disorders which occur at an Increased Frequency among Relatives of People with ALS

8.1. Introduction

This chapter does not directly address any of the aims described in Chapter 3, but attends to pre-requisite work for Project Aims 2.3 and 2.4. In order to delineate what degree of clustering of clinical disorders within families should be considered significant, it was first required to identify which disorders occur at an increased frequency among relatives of ALS probands. This was achieved through the conduction of two sub-studies:

1. A population-based case-control family aggregation study was performed for all ALS incident cases diagnosed between 1st January 2015 to 31st December 2017.

2. A pooled analysis was performed of data collected from the three separate family aggregation studies conducted over a ten-year period (2008-2017).

The former was conducted to expand on the range of neuropsychiatric disorders, and particularly neurodevelopmental disorders, assessed among relatives of probands in the previous family aggregation studies. The latter study offered enhanced power to explore further the relationship between ALS risk and the risk of other disorders among kindreds.

8.2. Methods

Recruitment procedures, inclusion/exclusion and data collection processes for each family aggregation study are outlined in Chapter 4. For the pooled analysis, the family history of only one ALS proband per kindred was considered. For both sub-studies, comparison was made for demographic and clinical features (where relevant) between proband cohorts and 1) ALS patients who did not participate in the study, diagnosed during the same time period and 2) control cohort. Kindred size and age and sex profile of relatives for both ALS probands and controls were compared. For the pooled analyses, data were compiled if available for two or more studies. Variables assessed included ALS, FTD, dementia (unspecified), Parkinson’s disease, multiple sclerosis, schizophrenia, bipolar affective disorder, autism, depression, suicide, alcohol dependence syndrome and unspecified neuropsychiatric disorders.
Prior to conduction of the pooled analysis, potential sources of heterogeneity between studies were explored. All family aggregation studies were conducted within the same group, under the direction of supervisor (OH). Standardised operating procedures for data collection and coding (aligning with DSM-IV criteria) were followed. While additional variables were assessed in the latter studies, core variables were gathered across all studies.

8.3. Results of Family Aggregation Study 2015-2017

8.3.1. Study participants and non-participants: Demographic and clinical characteristics

100 ALS probands who participated in the study were younger at age of onset and diagnosis (mean age of onset 61.8 years [95% CI 59.5, 64.1], mean age of diagnosis 63.2 years [95% CI 60.9, 65.5]) than non-participants diagnosed during the same time period (mean age of onset 65.1 years [95% CI 63.9, 66.3], [p=0.013]; mean age of diagnosis 66.7 years [95% CI 65.5, 67.9], [p=0.007]) (Table 8-1). Study participants were also more likely to have spinal onset disease compared to non-participants (79.0% v 56.6%, p=0.0002). No differences in sex composition or proportion carrying the pathogenic C9orf72 repeat expansion between the groups were observed (p=0.15).

Table 8-1: Demographic and clinical details of patients included or not included in Family Aggregation Study 2015-2017

<table>
<thead>
<tr>
<th></th>
<th>Patients included in study (n=100)</th>
<th>Patients not included in study (n=351)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal variables (no., %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>68 (68.0)</td>
<td>211 (60.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>79 (79.0)</td>
<td>198 (56.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>El Escorial (definite or probable)</td>
<td>52 (69.3)</td>
<td>140 (79.1)</td>
<td>0.096</td>
</tr>
<tr>
<td>C9orf72 repeat expansion (positive)</td>
<td>6 (6.7)</td>
<td>15 (7.1)</td>
<td>0.92</td>
</tr>
<tr>
<td>Continuous variables (mean and 95% confidence interval)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>61.8 (59.5, 64.1)</td>
<td>65.1 (63.9, 66.3)</td>
<td>0.013</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>63.2 (60.9, 65.5)</td>
<td>66.7 (65.5, 67.9)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Motor site of onset: Patients not included (n=350). El Escorial classification at diagnosis: Patients included (n=75), Patients not included (n=177). C9orf72 repeat expansion: Patients included in study (n=89), Patients not included in study (n=212).
8.3.2. Demographic characteristics of ALS patients and controls

Data from 3210 first- and second-degree relatives from 100 ALS families were compared with data from 1486 first- and second-degree relatives from 50 control families. Probands were matched for age at assessment (mean age patients 64.3 [95% CI 62.0, 66.6], controls 62.0 years [95% CI 59.3, 64.7] p=0.23), although there were more males in the patient cohort (68.0% v 47.1%, p=0.012).

No difference in the mean number of first- and second-degree relatives per kindred was observed between cohorts (patients 32.1 [95% CI 29.6, 34.6], controls 29.9 [95% CI 26.9, 32.8], p=0.30). The largest kindred size observed was an ALS kindred in which the proband had 72 first- and second-degree relatives. 1573 (49.0%) relatives of ALS probands were female comparable with 758 (49.6%) relatives of controls (p=0.68). A greater proportion of relatives were deceased in control kindreds compared with ALS kindreds (ALS kindred 36% v control kindred 42%, p=0.0001), but no difference in the age profile of relatives still alive in both cohorts was detected (mean age patients 41.2 years [95% CI 40.1, 42.3], controls 39.6 years [95% CI 38.1, 41.1], p=0.099)

8.4. Results: Comparison of frequency of neurodegenerative and neuropsychiatric disorders in relatives of ALS probands compared with relatives of controls (Family Aggregation study [2015-2017])

The proportion of relatives with various neurodegenerative and neuropsychiatric disorders are compared by proband cohort in Table 8-2. An increased risk of developing ALS was observed among relatives of ALS probands (p<0.0001). No other discernible differences were detected after correcting for multiple comparisons.
Table 8-2: Comparison of frequency of neurological or neuropsychiatric disorders among relatives of ALS patients versus controls (Family Aggregation Study 2015-2017)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Relatives of ALS patients (n=3210)</th>
<th>Relatives of Controls (n=1486)</th>
<th>RR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>26 (0.8)</td>
<td>0 (0.0)</td>
<td>1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All dementia</td>
<td>113 (3.5)</td>
<td>58 (3.9)</td>
<td>0.9</td>
<td>0.52</td>
</tr>
<tr>
<td>Alzheimer’s dementia</td>
<td>44 (1.4)</td>
<td>27 (1.8)</td>
<td>0.8</td>
<td>0.25</td>
</tr>
<tr>
<td>FTD</td>
<td>4 (0.1)</td>
<td>0 (0.0)</td>
<td>1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Vascular dementia</td>
<td>1 (0.0)</td>
<td>1 (0.1)</td>
<td>0.5</td>
<td>0.53</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>6 (0.2)</td>
<td>3 (0.2)</td>
<td>0.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>23 (0.7)</td>
<td>6 (0.4)</td>
<td>1.8</td>
<td>0.20</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>0 (0.0)</td>
<td>1 (0.1)</td>
<td>1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Stroke</td>
<td>86 (2.7)</td>
<td>29 (2.0)</td>
<td>1.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>15 (0.5)</td>
<td>7 (0.5)</td>
<td>1.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Tourette’s syndrome</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Depression</td>
<td>64 (2.0)</td>
<td>35 (2.4)</td>
<td>0.8</td>
<td>0.42</td>
</tr>
<tr>
<td>Anxiety</td>
<td>15 (0.5)</td>
<td>16 (1.1)</td>
<td>0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Bipolar affective disorder</td>
<td>8 (0.2)</td>
<td>3 (0.2)</td>
<td>1.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Suicide</td>
<td>5 (0.2)</td>
<td>7 (0.5)</td>
<td>0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Social anxiety</td>
<td>2 (0.1)</td>
<td>0 (0.0)</td>
<td>1.0</td>
<td>0.34</td>
</tr>
<tr>
<td>Post-traumatic stress disorder</td>
<td>1 (0.0)</td>
<td>1 (0.1)</td>
<td>0.5</td>
<td>0.53</td>
</tr>
<tr>
<td>Obsessive compulsive disorder</td>
<td>16 (0.5)</td>
<td>5 (0.3)</td>
<td>1.5</td>
<td>0.64</td>
</tr>
<tr>
<td>Phobias</td>
<td>0 (0.0)</td>
<td>1 (0.1)</td>
<td>1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>All addiction</td>
<td>88 (2.7)</td>
<td>42 (2.8)</td>
<td>1.0</td>
<td>0.87</td>
</tr>
<tr>
<td>Alcohol dependence syndrome</td>
<td>78 (2.4)</td>
<td>36 (2.4)</td>
<td>1.0</td>
<td>0.99</td>
</tr>
<tr>
<td>Drug dependence</td>
<td>3 (0.1)</td>
<td>3 (0.2)</td>
<td>0.5</td>
<td>0.39</td>
</tr>
<tr>
<td>Behaviour disturbance</td>
<td>8 (0.2)</td>
<td>2 (0.1)</td>
<td>1.9</td>
<td>0.52</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>12 (0.4)</td>
<td>1 (0.1)</td>
<td>5.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Psychosis (unspecified)</td>
<td>1 (0.0)</td>
<td>1 (0.1)</td>
<td>0.5</td>
<td>0.53</td>
</tr>
<tr>
<td>Eating disorder</td>
<td>3 (0.1)</td>
<td>5 (0.3)</td>
<td>0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Personality disorder</td>
<td>3 (0.1)</td>
<td>1 (0.1)</td>
<td>1.4</td>
<td>0.78</td>
</tr>
<tr>
<td>Autism</td>
<td>10 (0.3)</td>
<td>5 (0.3)</td>
<td>0.9</td>
<td>0.88</td>
</tr>
<tr>
<td>Asperger’s syndrome</td>
<td>2 (0.1)</td>
<td>0 (0.0)</td>
<td>1.0</td>
<td>0.34</td>
</tr>
<tr>
<td>Learning disability</td>
<td>14 (0.4)</td>
<td>5 (0.3)</td>
<td>1.3</td>
<td>0.81</td>
</tr>
<tr>
<td>Attention deficit hyperactivity disorder</td>
<td>10 (0.3)</td>
<td>2 (0.1)</td>
<td>2.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Dyslexia</td>
<td>13 (0.4)</td>
<td>11 (0.7)</td>
<td>0.5</td>
<td>0.13</td>
</tr>
</tbody>
</table>
8.5. Results of Pooled Analysis of Family Aggregation Studies 2008-2017

8.5.1. Results: Assessment of heterogeneity between family aggregation studies

The demographic and clinical characteristics of the ALS probands included in each family aggregation study are described in Table 8-3. A greater number of participants were recruited per calendar year in the earlier two studies compared with the 2015-2017 cohort. Furthermore, a greater proportion of ALS probands who participated in the 2015-2017 family aggregation study had spinal onset disease (p=0.006). Otherwise, no inter-study differences in sex composition, El Escorial criteria and mean ages of onset and diagnosis were observed.

Table 8-3: Comparison of demographic and clinical details of patients included in each family aggregation study

<table>
<thead>
<tr>
<th></th>
<th>2015-2017 (n=100)</th>
<th>2012-2014 (n=127)</th>
<th>2008-2011 (n=172)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nominal variables (no., %)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>68 (68.0)</td>
<td>69 (54.3)</td>
<td>94 (54.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>79 (79.0)</td>
<td>81 (63.8)</td>
<td>103 (60.0)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>El Escorial (definite or probable)</td>
<td>52 (69.3)</td>
<td>87 (79.1)</td>
<td>131 (79.4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Continuous variables (mean and 95% confidence interval)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>61.8 (59.5, 64.1)</td>
<td>62.8 (61.0, 64.6)</td>
<td>63.1(61.4, 64.8)</td>
<td>0.56</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>63.2 (60.9, 65.5)</td>
<td>64.2 (62.3, 66.1)</td>
<td>63.9(62.2, 65.6)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

8.5.2. Comparison of family aggregation studies: Reported results

A descriptive comparison of the results reported by each family aggregation study is available in Table 8-4. Results of frequency of dementia and Parkinson’s disease among
relatives were not reported in the published manuscript for the second family aggregation study (2012-2014). Similarly, the first-family aggregation study did not report on the frequency of alcohol dependence syndrome among relatives in the published manuscript for brevity purposes. This study did not collect data on the prevalence of autism among relatives of probands.

Direct comparison of the results reported by the second family aggregation study (2012-2014) with other studies was limited, as the former also included 64 third-degree relatives. The frequencies of dementia, Parkinson’s disease and schizophrenia were similar for both ALS proband and control groups across the first (2008-2011) and third (2015-2017) family aggregation studies. The absence a significant difference with respect to schizophrenia risk in the third study likely reflects insufficient power to detect this effect. A higher proportion of suicide was observed among relatives of controls in the third (2015-2017) family aggregation study. Inspection of the raw data for both ALS patients and control cohorts in this study did not identify any obvious clustering within a kindred. It is notable that a higher frequency of depression among relatives of both cohorts was observed in the third family aggregation study, compared with the first. While standardised procedures were followed to ensure data was collected in a consistent manner across studies, it is possible that this observation reflects some level of information creep. The family aggregation studies however were consistent in finding no increased risk of depression among relatives of ALS probands versus controls.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Relatives of Patients</td>
<td>3210</td>
<td>2116*</td>
<td>4050</td>
</tr>
<tr>
<td>No. Relatives of Controls</td>
<td>1486</td>
<td>2139</td>
<td>5634</td>
</tr>
<tr>
<td>Dementia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected Relatives of Patients (no., %)</td>
<td>113 (3.5)</td>
<td>n/a</td>
<td>152 (3.8)</td>
</tr>
<tr>
<td>Affected Relatives of Controls (no., %)</td>
<td>58 (3.9)</td>
<td>n/a</td>
<td>186 (3.3)</td>
</tr>
<tr>
<td>p value</td>
<td>0.52</td>
<td>n/a</td>
<td>0.26</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected Relatives of Patients (no., %)</td>
<td>23 (0.7)</td>
<td>n/a</td>
<td>24 (0.6)</td>
</tr>
<tr>
<td>Affected Relatives of Controls (no., %)</td>
<td>6 (0.4)</td>
<td>n/a</td>
<td>35 (0.6)</td>
</tr>
<tr>
<td>p value</td>
<td>0.20</td>
<td>n/a</td>
<td>0.86</td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected Relatives of Patients (no., %)</td>
<td>64 (2.0)</td>
<td>35 (1.7)</td>
<td>24 (0.6)</td>
</tr>
<tr>
<td>Affected Relatives of Controls (no., %)</td>
<td>35 (2.4)</td>
<td>31 (1.4)</td>
<td>31 (0.6)</td>
</tr>
<tr>
<td>p value</td>
<td>0.42</td>
<td>0.59</td>
<td>0.89</td>
</tr>
</tbody>
</table>
### Suicide

<table>
<thead>
<tr>
<th></th>
<th>Affected Relatives of Patients (no., %)</th>
<th>Affected Relatives of Controls (no., %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 (0.2)</td>
<td>13 (0.6)</td>
<td>14 (0.3)</td>
</tr>
<tr>
<td>Affected Relatives of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>7 (0.5)</td>
<td>4 (0.2)</td>
<td>4 (0.0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.06</td>
<td>0.04</td>
<td>0.004</td>
</tr>
</tbody>
</table>

### Alcohol

<table>
<thead>
<tr>
<th></th>
<th>Affected Relatives of Patients (no., %)</th>
<th>Affected Relatives of Controls (no., %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>78 (2.4)</td>
<td>63 (2.9)</td>
<td>n/a</td>
</tr>
<tr>
<td>Affected Relatives of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>36 (2.4)</td>
<td>43 (2.0)</td>
<td>n/a</td>
</tr>
<tr>
<td>p value</td>
<td>0.99</td>
<td>0.045</td>
<td>n/a</td>
</tr>
</tbody>
</table>

### Schizophrenia/psychosis

<table>
<thead>
<tr>
<th></th>
<th>Affected Relatives of Patients (no., %)</th>
<th>Affected Relatives of Controls (no., %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 (0.4)</td>
<td>17 (0.8)</td>
<td>13 (0.3)</td>
</tr>
<tr>
<td>Affected Relatives of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>2 (0.1)</td>
<td>5 (0.2)</td>
<td>5 (0.0)</td>
</tr>
<tr>
<td>p value</td>
<td>0.11</td>
<td>0.02</td>
<td>0.009</td>
</tr>
</tbody>
</table>

### Autism

<table>
<thead>
<tr>
<th></th>
<th>Affected Relatives of Patients (no., %)</th>
<th>Affected Relatives of Controls (no., %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 (0.3)</td>
<td>10 (0.5)</td>
<td>n/a</td>
</tr>
<tr>
<td>Affected Relatives of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>5 (0.3)</td>
<td>1 (0.0)</td>
<td>n/a</td>
</tr>
<tr>
<td>p value</td>
<td>0.88</td>
<td>0.03</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*Includes 64 third-degree relatives

### 8.5.3. Results: Comparison of demographic and clinical characteristics

For the pooled analysis, the raw data from each study as pertaining to first and second-degree relatives only was analysed. Frequencies of neuropsychiatric and neurodegenerative disorders were compared, where at least two studies had collected data on the variable. Overall, the 399 probands in the pooled cohort represented 32.1% of all ALS patients diagnosed and registered with the Irish ALS Register during that time period (n=1242). Both study participants and non-participants were equally matched with respect to sex composition (included 56.4%; not included 56.5%, p=0.98), but there was a greater proportion with spinal onset disease in the cohort who participated in the study (included 264/375 [70.4%], not included 482/795 [60.6%], p=0.001). Those who participated were also younger at onset and diagnosis (mean age onset 62.5 years [95% CI 61.4, 63.6]; mean age diagnosis 63.7 years [95% CI 62.6, 64.8], p<0.0001) compared to non-participants (mean age onset 65.4 years [95% CI 64.6, 66.2]; mean age diagnosis 63.7 years [95% CI 66.0, 67.6] p<0.0001). Finally, among those in whom DNA samples were available for testing, a greater proportion of those who participated in the family aggregation study carried the pathogenic C9orf72 repeat expansion (44/342 [12.9%]) compared with non-participants (38/473 [8.0%]), p=0.024.

Compared with control probands (n=374), the pooled ALS probands showed no differences in sex composition or mean age at assessment (sex [p=0.46], age at assessment [p=0.47]). Furthermore, there was no difference in the mean number of first- and second-degree relatives in ALS kindreds compared with control kindreds (23.3 v 24.7 persons, p=0.41).
8.6. Results: Comparison of frequency of neurodegenerative and neuropsychiatric disorders in relatives of ALS probands compared with relatives of controls (Pooled Family Aggregation Study [2008-2017])

The relatives of ALS probands were found to be at increased risk for developing several neuropsychiatric disorders compared with relatives of controls, including ALS, FTD, schizophrenia, bipolar affective disorder, suicide, alcohol dependence syndrome and unspecified neuropsychiatric disorders (Table 8-5). A greater proportion of relatives from kindreds with familial clustering of ALS, compared with those from sporadic ALS kindreds, had alcohol dependence syndrome (2.6% v 1.1%, p=0.045), suicide (0.7% v 0.2%, p=0.005) or unspecified neuropsychiatric disorders (0.9% v 0.3%, p=0.025). No difference in risk between relative cohorts were observed for dementia (unspecified), Parkinson’s disease, multiple sclerosis and depression. However, after controlling for multiple comparisons, only ALS and schizophrenia were confirmed to be associated with an increased risk in relatives of ALS probands.

<table>
<thead>
<tr>
<th>Affected relatives (no., %)</th>
<th>Relatives of ALS patients (n=9312)</th>
<th>Relatives of Controls (n=9248)</th>
<th>RR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>90 (1.0)</td>
<td>8 (0.1)</td>
<td>11.2</td>
<td>(5.4, 23.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Depression</td>
<td>124 (1.3)</td>
<td>100 (1.1)</td>
<td>1.2</td>
<td>(0.9, 1.6)</td>
<td>0.12</td>
</tr>
<tr>
<td>Bipolar affective disorder</td>
<td>17 (0.2)</td>
<td>7 (0.0)</td>
<td>2.4</td>
<td>(1.0, 5.8)</td>
<td>0.049</td>
</tr>
<tr>
<td>Suicide</td>
<td>32 (0.3)</td>
<td>15 (0.2)</td>
<td>2.1</td>
<td>(1.1, 3.9)</td>
<td>0.016</td>
</tr>
<tr>
<td>Alcohol dependence syndrome</td>
<td>167 (1.8)</td>
<td>124 (1.3)</td>
<td>1.3</td>
<td>(1.1, 1.7)</td>
<td>0.013</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>32 (0.3)</td>
<td>9 (0.1)</td>
<td>3.5</td>
<td>(1.7, 7.4)</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
8.6.1. Results: Impact of C9orf72 repeat expansion

The impact of the C9orf72 status of the ALS proband on the risk of neurodegenerative and neuropsychiatric disorders among relatives was assessed. Only disorders shown to occur at a significantly increased frequency in ALS kindreds in the prior analysis were compared. FTD risk among relatives was assessed due to the known causal link with the pathogenic repeat expansion. An increased risk of FTD was observed in relatives of ALS proband who carried the C9orf72 repeat (RR 123.9 [95% CI 6.9, 2238.9]) and also in relatives of non-carrier probands (170.4 [95% CI 9.7, 2979.5]) compared with controls (Table 8-6). In contrast, the increased risk of schizophrenia was only seen in relatives of C9orf72 positive probands (5.7 [95% CI 1.1, 30.9]).

Table 8-6: Relative risk of schizophrenia and FTD in relatives of C9orf72 positive and negative ALS probands

<table>
<thead>
<tr>
<th>Disease</th>
<th>Relatives</th>
<th>RR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizophrenia</td>
<td>Relatives of C9orf72 positive proband</td>
<td>5.7</td>
<td>(1.1, 30.9)</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9orf72 negative proband</td>
<td>1.2</td>
<td>(0.4, 3.6)</td>
<td>0.68</td>
</tr>
<tr>
<td>FTD</td>
<td>Relatives of C9orf72 positive proband</td>
<td>123.9</td>
<td>(6.9, 2238.9)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9orf72 negative proband</td>
<td>170.4</td>
<td>(9.7, 2979.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
8.7. Discussion

The pooled analysis of three Irish population-based family aggregation studies is the largest analysis conducted to date examining the risk of neurodegenerative and neuropsychiatric disorders among ALS kindreds. The findings that multiple neuropsychiatric disorders occur at an increased frequency in relatives of relatives of ALS probands highlights the phenotypic heterogeneity associated with the ALS genetic risk profile. In particular, this work supports a strong epidemiological link between ALS, FTD and schizophrenia risk which has previously been shown to be driven by both rare variants of large effect and to a lesser extent by common smaller effect variants.

A borderline significantly increased risk of unspecified neuropsychiatric disorders was also observed among ALS kindreds. Review of the raw data revealed that in many instances these unspecified disorders were characterised by atypical psychotic episodes not meeting criteria for schizophrenia, suggesting that the link between ALS and psychotic like disorders may be conservatively estimated in this study. Notably, both the risk of bipolar affective disorder and autism were initially suggested as increased in relatives of ALS probands, although this did not withstand correction for multiple comparisons. Bipolar affective disorder, autism and schizophrenia are strong genetically driven disorders, which share significant overlap in common risk variants resulting in dysfunction neural development and neuronal maintenance. Demonstrating genetic linkage between ALS and these disorders supports the interesting viewpoint that some forms of ALS should be considered as a neurodevelopmental disorder, similar to these conditions.

By contrast, both suicide and alcohol dependence syndrome were also initially found to occur at a higher frequency in relatives of ALS probands, particularly among relatives of those with familial ALS. Again, this did not withstand correction for multiple comparisons. These disorders are reported to be moderately heritable, with both believed to mediated in part through inheritance of liable personality traits (impulsive aggression, anti-socialism, neurotism). Yet, both disorders are also believed to have strong familial non-genetic components (intra-familial culture, imitation, abuse etc), which makes it difficult to distinguish the relative importance of genetic mediated versus familial mediated clustering.

Finally, previous studies in different populations have reported on an increased incidence of Parkinson’s disease and dementia among relatives of ALS patients, which is not supported by the findings of this pooled analysis. A large Swedish register-based case-
control study by Longinetti et al (2017)\(^{356}\) reported on an increased risk of neurodegenerative disorders (encompassing FTD, Alzheimer’s dementia, unspecified dementia and Parkinson’s disease) among siblings of ALS probands, but not parents or children. In contrast, an increased risk of psychiatric disorders (encompassing schizophrenia, bipolar disorder, depression, neurotic disorders, stress-related disorders and alcohol and/or drug abuse/dependence) was observed among children of patients with ALS, but not parents or siblings of those with ALS. Differences in neuropsychiatric risk in the different relative groups in this study may reflect changing probabilities of being diagnosed with a psychiatric disorder over their lifetimes of each cohort.

Similarly, the generational difference with respect to neurodegenerative diseases likely reflect a cohort effect where siblings are reaching an age where they are more likely to develop neurodegenerative disorders or indeed feasibly may be misdiagnosed as such during the early stages of developing ALS. The same study by Longinetti et al (2017)\(^{356}\) demonstrated that ALS patients were at a 10-fold increased risk of being diagnosed with Parkinson’s disease or dementia in the year prior to ALS diagnosis. These likely reflect initial misdiagnoses in the context of atypical presentations of ALS which is an important potential confounder to account for when examining the phenotypic presentations within ALS kindreds. Nonetheless, our pooled analysis showed stronger support for an overlap between ALS and neuropsychiatric disorders which typically manifest among those of a younger age profile. It did not support a link between ALS and other neurodegenerative disorders, excluding FTD. As such, the potential for misdiagnoses is less likely to be a source of concern.

The findings of the pooled analysis of family aggregation studies, align with those from a similar but smaller study by Devenney et al (2019)\(^{355}\) which compared the frequency of neuropsychiatric disorders among relatives of 29 C9orf72 repeat expansion probands with those of 60 non-carriers probands. The authors observed a similar increased risk of schizophrenia among relatives of C9orf72 carriers, with a suggestion of an increased risk of suicide and autism among C9orf72 kindreds, although the latter disorders were of borderline significance. While the findings of that study are concordant with those from the pooled analysis, it is not possible to directly compile the results as the former study did not employ a control relative cohort. As described in Chapter 2, the use of non-carrier relatives as a comparison group offers certain advantages but forsakes an opportunity to also explore the phenotypic profile of this cohort.

Indeed, the pooled family aggregation analysis demonstrated that relatives of C9orf72 negative ALS probands were also at increased risk of developing FTD. Several other
genetic mutations are known to link ALS and FTD, as discussed in Chapter 1, no ALS probands in the pooled analysis study carried other known FTD associated genes, which suggests that other rare, large effect pleiotropic genes variants linking both disorders may potentially exist within some of these isolated kindreds. Most importantly, this observation highlights that, while the C9orf72 repeat expansion is undoubtedly highly important in terms of ALS risk and its neuropsychological manifestations, by focusing solely on this mutation, the opportunity is missed to explore to what extent phenotypic variance within kindreds may be attributable at least in part to as-of-yet unidentified novel gene mutations or important gene-gene interactions.

8.7.1. Limitations

The greatest strength of this study is the large population size assessed using a population-based approach with medical and psychiatric history information collected on over 18500 relatives of close to 400 ALS patients and 374 controls over a ten-year period. While it is known that simple pooled analyses of separate studies may yield spurious results if they do not account for differences in co-variates and study quality, size and procedures\(^{524}\), this approach was chosen for this study as all family aggregation studies were conducted by the same group, under the same supervisor and followed the same study procedures to conduct population-based studies of the same overall population. A meta-analytic approach would have resulted in exclusion of the second family aggregation study, as data from third-degree relatives were also included in the final published results. Furthermore, the two other studies\(^{355, 356}\) assessing the frequency of neurodegenerative and neuropsychiatric disorders among ALS kindreds used different methodological approaches or only assessed specific cohorts, and so their data were not suitable for amalgamation. As the raw from all three Irish family aggregation studies were available to this author, a pooled analysis of first- and second-degree relatives only was confirmed as feasible after exploration for sources of heterogeneity between studies.

The probands included in the pooled analysis were younger and a greater proportion carried the C9orf72 repeat than Irish ALS patients diagnosed during the study period who did not participate in the study. Older patients, who often physically decline from the disorder quicker may be less likely to participate in research so it remains unclear as to how generalizable these findings are to that cohort of patients. In contrast, the slight enrichment of probands carrying the C9orf72 repeat expansion likely reflects a heightened awareness of the impact of familial clustering of disease and so these probands may be potentially more likely to participate in this type of research study. Nonetheless, for comparison purposes no differences between the pooled patient and
control cohorts in respect to sex and age at assessment of the proband were detected. Similarly, no differences in kindred size or age profile of relatives were detected between both cohorts.

The only notable source of heterogeneity between studies was the greater proportion of spinal onset disease among ALS probands in the 2015-2017 family aggregation study. In that study, the evidence of increased neuropsychiatric disorders among ALS kindreds was less apparent, which may reflect the reduced power in the analysis, stemming from the lower number of probands recruited to this specific study. Both factors may be tied together by considering the lower proportion of C9orf72 positive ALS probands which resulted as probands from kindreds included in previous family aggregation studies were not included in 2015-2017 study. C9orf72 positive carriers are more likely to present with bulbar or cognitive onset disease and have a high incidence of neuropsychiatric disorders among relatives.

This study is further limited in that all medical and psychiatric information collected about relatives was through proxy-report by the proband. Probands with a greater proportion of younger relatives may be more likely to report on certain diagnoses in these relatives (e.g., autism in grandchildren), due to the increasing prevalence of these diagnoses in younger cohorts\(^5\). Equally, older relatives who were more recently diagnosed may receive a more specific diagnosis (e.g., FTD instead of unspecified dementia). While both scenarios are potential sources of ascertainment bias, no differences in the age profile of relatives between cohorts were observed. An alternative family study method approach offers enhanced accuracy with respect to diagnoses in relatives as each relative is contacted directly and provides their own medical history data. Diagnoses can be further confirmed through physical examination of the relatives and review of their medical records. This approach however is labour intensive and expensive.

Comparable results with respect to the sensitivity and specificity of diagnoses in relatives can be achieved using the family history method\(^5\) employed in the three family aggregation studies assessed here. This approach offers the advantage of allowing for the collection of data on large numbers of relatives. Collecting data directly on the same number of relatives assessed in the pooled analysis would have been prohibitively unfeasible. Nonetheless, while this approach offers significant power advantages through the sheer numbers of relatives assessed, enhanced power can also be achieved through detailed phenotyping of informative kindreds as discussed in Chapters 10, 11 and 12. Direct neuropsychiatric, neurocognitive and behavioural assessment of relatives using standardised clinical batteries may detect subtle clinical changes that do not meet
current diagnostic criteria. Identifying such changes will hopefully advance our understanding of the shared pathobiological processes underpinning these disorders.

8.7.2. Conclusion

In this chapter, I have reported on the largest population-based family aggregation study conducted to date examining the risk of neurodegenerative and neuropsychiatric disorders among ALS kindreds. This work highlights the extent of phenotypic heterogeneity associated with the ALS genetic risk profile, as evidenced by an increased risk in relatives of ALS probands of several neuropsychiatric disorders including ALS, FTD, schizophrenia, bipolar affective disorder, suicide, alcohol dependence syndrome and unspecified neuropsychiatric disorders. In particular, this work supports a strong epidemiological link between ALS, FTD and schizophrenia risk, but refutes an association between ALS and unspecified dementia, Parkinson’s disease, multiple sclerosis and depression. This work provides a foundational basis allowing for the exploration of the significance of different familial clustering patterns in ALS. Ultimately this avenue can be pursued further, to determine the nature of the relationship between neuropsychiatric clustering in families and the phenotypic presentation in ALS probands.
9. Chapter 9: Results Part V: Modelling Familial Clustering within ALS Kindreds

Published Work List

The work described in section 9.3 has been published in the peer-reviewed journal Neurology Genetics as:

9.1. Introduction

The findings from the pooled family aggregation analysis strongly suggest that familial ALS classification criteria should be expanded to incorporate the presence of certain neuropsychiatric phenotypes within ALS kindreds. Indeed, the temporal analysis of familial ALS incidence suggests that this approach has been already adopted to some degree. Still, there remains a lack of clarity as to what exactly constitutes significant familial clustering with respect to these expanded phenotypes. In this chapter, I employ two probability models to delineate when the occurrence of clustering of clinical disorders within families exceeds that expected by chance alone (Sub-Aim 2.3). Finally, using data from the Irish ALS Register, I explore the clinical relevance of expanding how we define familial ALS to encompass other neuropsychiatric disorders (Sub-Aim 2.4).

9.2. Methods

Our group have previously shown that the likelihood of FALS increases based in the number of ALS patients within a kindred, and the size of the extended kindred. Using the same approach, the exact probability of a relative developing other neurodegenerative or neuropsychiatric disorders for a given kindred size, may be calculated. Similarly, using the average rate of familial clustering for different disorders obtained from the pooled family aggregation studies, the probability of relatives developing associated disorders may be calculated without need for knowledge of the proband’s kindred size. In these ways, what should be considered as significant familial clustering linked to ALS can be defined.

To examine the clinical relevance of expanding the definition of familial ALS, a cohort study was performed comparing probands with classical familial ALS, expanded forms of familial ALS and sporadic ALS cases. Data from the Irish ALS Register from 1995 to 2019 were reviewed and all ALS cases meeting classical and expanded familial ALS and sporadic ALS criteria as defined below were identified. These criteria were selected based on the results of the modelling studies, with sporadic cases defined by the absence of potentially significant neurodegenerative and psychiatric disorders (as per the pooled family aggregation analysis). Individuals diagnosed with PLS, PMA and Kennedy’s disease were excluded from the analysis. DNA results for all cases were obtained from the Irish ALS DNA biobank, where available. Demographic and clinical variables assessed included sex, site of onset, C9orf72 status, age at onset and diagnosis and survival from onset and diagnosis, with censor date set as 31st December 2019.
Results

9.3. Model 1: The Binomial Distribution Model

The binomial distribution model may be used to determine the probability of observing a certain number of pre-specified outcomes in a specific number of trials. It is used to model probability when there are two possible outcomes. Therefore, it is useful for modelling probability in the medical field where a person may be defined as either having a certain disease or not. In this model, the occurrence of the outcome of interest is labelled as a “success”. Using the reported lifetime adjusted individual risks of developing ALS\(^{515}\), FTD\(^{527}\) and schizophrenia\(^{528}\), the probability of an individual, within a given kindred size, developing each disorder was calculated separately (Tables 9-2,3,4). FTD and schizophrenia were chosen as they have the strongest evidence base supporting genetic linkage with ALS. The probability of having a relative with dementia (high prevalence disorder) was modelled for comparison purposes\(^{529}\) (Table 9-5).

Binomial Distribution Formula

\[
P(x) = \binom{n}{x} p^x q^{n-x} = \frac{n!}{(n-x)!x!} p^x q^{n-x}
\]

\(n\): number of trials. \(x\): number of successes desired. \(p\): probability of success in one trial. \(q\): probability of failure in one trial \((q=1-p)\).

In determining the probability that a relative in a given kindred may develop a disorder, the assumptions of the binomial distribution model are met.

- The number of observations is fixed.
- Each trial has only two possible outcomes.
- Each trial is independent of the previous trials.
- The probability of success remains constant for each trial.

At a given point in time, the number of relatives in a kindred can be considered a fixed entity. Each relative may develop the disorder or not. One relative developing ALS, FTD or schizophrenia is not believed to directly influence another relative’s risk of developing the disorder. Finally, while the relative risk of developing a disorder may chance over an individual’s lifetime, the overall lifetime risk in a given population is fixed.
9.3.1. Model findings

Using these analyses, it is calculated that a kindred of 35 will introduce a 5% probability of having a relative with FTD by chance alone, but that the presence of 2 or more relatives with FTD is sufficiently unlikely by chance (p=0.025) to provide a credible criterion for FALS (Table 9-1). Similarly, there is a 5% probability of having one relative with schizophrenia in the presence of at least 13 unaffected relatives, with a diminishing probability of having 2 or more first- or second-degree relatives with schizophrenia within extended kindreds, rendering the presence of 2 or more family members with schizophrenia a credible criterion by which to extend the definition of FALS. The finding that a kindred size of 19 introduces a 5% chance of one member developing ALS supports the work by Byrne et al (2011) who reported, that in Ireland where the average family size was 17 members, 1 in 25 people when asked would have a family history of ALS purely by chance. Finally, assuming a lifetime risk of developing dementia in those over 65 of approximately 1 in 5, the presence of dementia in 2 relatives within an ALS kindred is insufficient to make a diagnosis of likely FALS, irrespective of family size. In this high prevalence disorder, only kindreds with familial clustering where approximately half the relatives develop dementia could be considered significant.

Table 9-1: Kindred size at or above which the probability of member(s) developing a disorder exceeds 5%

<table>
<thead>
<tr>
<th>Number of affected relatives</th>
<th></th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>19</td>
<td>139</td>
<td>332</td>
</tr>
<tr>
<td>FTD</td>
<td>35</td>
<td>267</td>
<td>640</td>
</tr>
<tr>
<td>SCZ</td>
<td>13</td>
<td>93</td>
<td>221</td>
</tr>
<tr>
<td>Dementia</td>
<td>n/a</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

n/a: not applicable. The numbers in the cells relate to the minimum kindred size at or above which an individual member may be expected to develop a disorder based on chance alone. For example, a kindred of 19 will introduce a 5% probability of having one relative with ALS by chance alone.
### Table 9-2: Probability of having exactly this number of relatives with ALS

<table>
<thead>
<tr>
<th>Family size</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>7</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>8</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>11</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>12</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>13</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>14</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>15</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>16</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>17</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>18</td>
<td>95%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>19</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>20</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Based on lifetime risk of ALS in general population of 1 in 385 people\textsuperscript{515}

### Table 9-3: Probability of having exactly this number of relatives with FTD

<table>
<thead>
<tr>
<th>Family size</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>7</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>8</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>11</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>12</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>13</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>14</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>15</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>16</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>17</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>18</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>19</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>20</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Based on lifetime risk of FTD in general population of 1 in 742 people\textsuperscript{527}
### Table 9-4: Probability of having exactly this number of relatives with schizophrenia

<table>
<thead>
<tr>
<th>Family size</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>99%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>7</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>8</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>10</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>11</td>
<td>96%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>12</td>
<td>95%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>13</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>14</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>15</td>
<td>94%</td>
<td>6%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>16</td>
<td>94%</td>
<td>6%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>17</td>
<td>94%</td>
<td>6%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>18</td>
<td>93%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>19</td>
<td>93%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>20</td>
<td>92%</td>
<td>7%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Based on lifetime risk of schizophrenia in general population of 1 in 256.528

### Table 9-5: Probability of having exactly this number of relatives with dementia (unspecified)

<table>
<thead>
<tr>
<th>Family size</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82%</td>
<td>18%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>67%</td>
<td>30%</td>
<td>3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>55%</td>
<td>37%</td>
<td>8%</td>
<td>1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>45%</td>
<td>40%</td>
<td>13%</td>
<td>2%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>37%</td>
<td>41%</td>
<td>18%</td>
<td>4%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>30%</td>
<td>40%</td>
<td>22%</td>
<td>7%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>7</td>
<td>25%</td>
<td>38%</td>
<td>25%</td>
<td>9%</td>
<td>2%</td>
<td>0%</td>
</tr>
<tr>
<td>8</td>
<td>20%</td>
<td>36%</td>
<td>28%</td>
<td>12%</td>
<td>3%</td>
<td>1%</td>
</tr>
<tr>
<td>9</td>
<td>16%</td>
<td>33%</td>
<td>29%</td>
<td>15%</td>
<td>5%</td>
<td>1%</td>
</tr>
<tr>
<td>10</td>
<td>13%</td>
<td>30%</td>
<td>30%</td>
<td>18%</td>
<td>7%</td>
<td>2%</td>
</tr>
<tr>
<td>11</td>
<td>11%</td>
<td>27%</td>
<td>30%</td>
<td>20%</td>
<td>9%</td>
<td>3%</td>
</tr>
<tr>
<td>12</td>
<td>9%</td>
<td>24%</td>
<td>29%</td>
<td>22%</td>
<td>11%</td>
<td>4%</td>
</tr>
<tr>
<td>13</td>
<td>7%</td>
<td>21%</td>
<td>28%</td>
<td>23%</td>
<td>13%</td>
<td>5%</td>
</tr>
<tr>
<td>14</td>
<td>6%</td>
<td>19%</td>
<td>27%</td>
<td>24%</td>
<td>15%</td>
<td>7%</td>
</tr>
<tr>
<td>15</td>
<td>5%</td>
<td>16%</td>
<td>26%</td>
<td>25%</td>
<td>16%</td>
<td>8%</td>
</tr>
<tr>
<td>16</td>
<td>4%</td>
<td>14%</td>
<td>24%</td>
<td>25%</td>
<td>18%</td>
<td>10%</td>
</tr>
<tr>
<td>17</td>
<td>3%</td>
<td>12%</td>
<td>22%</td>
<td>25%</td>
<td>19%</td>
<td>11%</td>
</tr>
<tr>
<td>18</td>
<td>3%</td>
<td>11%</td>
<td>20%</td>
<td>24%</td>
<td>20%</td>
<td>13%</td>
</tr>
<tr>
<td>19</td>
<td>2%</td>
<td>9%</td>
<td>19%</td>
<td>23%</td>
<td>21%</td>
<td>14%</td>
</tr>
<tr>
<td>20</td>
<td>2%</td>
<td>8%</td>
<td>17%</td>
<td>23%</td>
<td>21%</td>
<td>15%</td>
</tr>
</tbody>
</table>

Based on lifetime risk of dementia in general population of 1 in 5 people.529
9.4. Model 2: The Poisson Distribution Model

The Poisson distribution is a discrete probability distribution that expresses the probability of a given number of events occurring randomly in a given interval of time or space. It may be derived as a limiting form of the binomial distribution as the number of trials goes to infinity and probability of success in each trial is very low. As such, it may be useful for modelling the probability of familial clustering of a rare disease. In comparison with the binomial distribution model, it does not rely on knowing the exact number of trials. In this respect, the Poisson distribution model can be used to answer the question “What is the probability of having X number of relatives with a specified disorder?” as opposed to the binomial model which answers the question “What is the probability of having X number of relatives with a specified disorder, in a kindred size of Y?”. The Poisson model allows for a simple heuristic approach to defining significant familial clustering, that does not require knowledge of the exact number of relatives in a kindred.

Instead, the Poisson model depends on knowing the average rate of occurrence of events in a given interval of time or space. Using data from the pooled Irish population-based family aggregation studies, conducted over a 10-year period, it is possible to determine the average number of relatives with specified disorders reported by a proband. The average number of first- and second-degree relatives with ALS, FTD and schizophrenia reported by ALS probands was calculated by dividing the total number of relatives (of each category) with each disorder by the number of probands providing family history information (Table 9-6).

<table>
<thead>
<tr>
<th></th>
<th>No. affected relatives</th>
<th>Average number of occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. probands</td>
<td>FDR</td>
</tr>
<tr>
<td>ALS</td>
<td>399</td>
<td>47</td>
</tr>
<tr>
<td>FTD</td>
<td>272*</td>
<td>7</td>
</tr>
<tr>
<td>SCZ</td>
<td>399</td>
<td>15</td>
</tr>
<tr>
<td>ALS or FTD</td>
<td>272*</td>
<td>30</td>
</tr>
<tr>
<td>ALS or SCZ</td>
<td>399</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 9-6: Total and average number of affected relatives with specified disorders, from pooled family aggregation study (2008-2017)
ALS: amyotrophic lateral sclerosis. FDR: first-degree relative. FTD: frontotemporal dementia. SCZ: schizophrenia. SDR: second-degree relative. $\lambda$: mean number of occurrences in the interval (i.e., the number of affected relatives as a proportion of the number of probands providing family history information. *Data on FTD among relatives was available only for family aggregation studies (2012-2014) and (2015-2017) with combined number of ALS probands assessed ($n=272$). 

Poisson Distribution Formula

$$P(X = x) = \frac{\lambda^x e^{-\lambda}}{x!}$$

$e$: Euler’s constant ($\approx 2.71828$); $\lambda$: mean number of occurrences in the interval; $x = 0, 1, 2, 3, \ldots$

The following assumptions of the Poisson distribution model are considered when determining the probability of relative in a kindred developing a specified disorder

- The probability of two or more occurrences of the event at the same instant is negligible.
- The average rate at which events occur is constant and independent of the occurrence of any event.
- The probability of an event occurring in a given population is proportional to the size of the population.

As ALS, FTD and schizophrenia are all relatively rare disorders, the probability of co-occurrence of multiple disorders at the same instance is negligible. As discussed above, the probability of individual developing ALS, FTD or schizophrenia is not believed to directly increase as a result of exposure to a relative with these conditions. Finally, as demonstrated in the binomial model above, the probability of an event occurring within a kindred to proportional to the size of the kindred. In the pooled family aggregation study analysis, the average kindred size for ALS probands was approximately 23 first- and second-degree relatives. While it is possible that the average kindred size may change with time, the long time-period over which the data was collected and the late-onset nature of ALS, suggests that it is reasonable to believe that this average is a reasonable estimate for kindred size for current ALS patients and those diagnosed in the near future.
9.4.1. Model Findings

These analyses demonstrate that there is a less than 5% chance of an ALS proband reporting a family history of FTD in a first- or second-degree relative, providing a credible criterion for FALS (Table 9-7). Similarly, an ALS proband reporting of one or more first-degree relatives or two or more first- or second-degree relatives with schizophrenia is unlikely to be a chance occurrence and should be considered to represent significant familial clustering of disease. For ALS probands reporting a family history of ALS, only those with two or more first- or second-degree relatives with ALS should be considered representative of significant clustering. This is consistent with Byrne criteria for "Definite familial ALS" and aligns with the views of the vast majority of respondents to a survey on ALS genetic testing practices worldwide, where most said they would offer genetic testing to the proband who provided a similar family history of disease. Finally, for those not meeting this classical definition of familial ALS, the presence of one first- or second-degree relative with ALS along with an additional first- or second-degree relative with either FTD or schizophrenia was sufficiently unlikely to occur by chance alone and as so also provides a credible criterion for FALS.

Summary of findings: Significant familial clustering of disorders in ALS probands

- **FTD**: ≥1 first- or second-degree relative with FTD
- **Schizophrenia**: ≥1 first-degree relative with schizophrenia OR ≥ 2 first- or second-degree relatives with schizophrenia
- **ALS**: ≥ 2 first or second-degree relatives with ALS OR 1 first- or second-degree relative with ALS and another first- or second-degree relative with FTD or schizophrenia
Table 9-7: Poisson distribution for probability of first- and/or second-degree relative developing ALS, FTD or schizophrenia

<table>
<thead>
<tr>
<th>No. of affected relatives</th>
<th>ALS</th>
<th>FTD</th>
<th>Schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FDR</td>
<td>SDR</td>
<td>FDR or SDR</td>
</tr>
<tr>
<td>0</td>
<td>0.888</td>
<td>0.897</td>
<td>0.798</td>
</tr>
<tr>
<td>1</td>
<td>0.104</td>
<td>0.096</td>
<td>0.180</td>
</tr>
<tr>
<td>2</td>
<td>0.006</td>
<td>0.005</td>
<td>0.020</td>
</tr>
<tr>
<td>No. of affected relatives</td>
<td>ALS or FTD</td>
<td>ALS or Schizophrenia</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FDR</td>
<td>SDR</td>
<td>FDR or SDR</td>
</tr>
<tr>
<td>0</td>
<td>0.895</td>
<td>0.860</td>
<td>0.770</td>
</tr>
<tr>
<td>1</td>
<td>0.009</td>
<td>0.129</td>
<td>0.201</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>0.009</td>
<td>0.026</td>
</tr>
</tbody>
</table>
Clinical importance of expanded familial ALS kindreds

Before expanding the definition of familial ALS to include significant clustering of associated neuropsychiatric disorders, the clinical implications of such an expansion were explored, with significant clustering defined as per the results of the modelling studies above. The clinical features of probands from kindreds with familial clustering of 1) ALS only (n=65), 2) ALS and FTD (n=22), 3) ALS and SCZ (n=48) and sporadic ALS patients (n=1814) were compared (Table 9-8). The former category consisted of 65 classical familial ALS probands from 32 distinct kindreds with each ALS proband having at least two first- or second-degree relatives with ALS (‘Definite Familial ALS’). While these kindreds also included some relatives with FTD and schizophrenia, other kindreds without predominance of ALS but with significant clustering of FTD or schizophrenia were considered separately. These ‘Expanded familial ALS with FTD’ and ‘Expanded familial ALS with schizophrenia’ kindreds would not have been considered as higher genetic risk kindreds, except through the proposed expanded definitions of familial ALS modelled above. A breakdown of the composition of ‘Expanded familial ALS with FTD’ and ‘Expanded familial ALS with schizophrenia’ kindreds are provided in Appendix 3.

Allowing for differences in respect to cohort size, considerable variance in respect to age of onset and diagnosis was observed between probands from the different cohorts (p<0.001). All familial cohorts shared similar mean ages of onset (classical FALS 58.3 years [95% CI 55.9, 60.7], expanded FALS [schizophrenia] 60.5 years [95% CI 57.0, 63.9], expanded FALS [FTD] 59.8 years [95% CI 56.2, 63.4], p=0.54) and mean ages of diagnosis (classical FALS 59.8 years [95% CI 57.5, 62.2], expanded FALS [schizophrenia] 61.9 years [95% CI 58.5, 65.5], expanded FALS [FTD] 61.1 years [95% CI 57.3, 64.9], p=0.54). Both classical familial ALS and expanded familial ALS with schizophrenia cohorts were significantly younger at onset than those with sporadic ALS (mean age of onset 65.1 years [95% CI 64.5, 65.7], p<0.001 and p=0.040 respectively). Those with classical familial ALS were also significantly younger at diagnosis than those with sporadic ALS (mean age of diagnosis 66.3 years [95% CI 65.8, 66.9], p<0.001), with a trend towards significance noted in those with expanded familial ALS with schizophrenia (p=0.064) (Figure 9-1). Mean ages of onset and diagnosis were not found to differ significantly between those with sporadic ALS and expanded familial ALS with FTD (mean age onset p=0.201, mean age diagnosis p=0.217). No differences in sex composition, motor site of onset or survival between cohorts were observed.
Table 9-8: Comparison of demographic and clinical characteristics by familial status

<table>
<thead>
<tr>
<th></th>
<th>Classical FALS (n=65)</th>
<th>Expanded FALS (SCZ) (n=48)</th>
<th>Expanded FALS (FTD) (n=22)</th>
<th>SALS (n=1814)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nominal variables (no., %)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>33 (50.8)</td>
<td>27 (56.3)</td>
<td>13 (59.1)</td>
<td>1021 (56.3)</td>
<td>0.84</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>44 (68.8)</td>
<td>32 (66.7)</td>
<td>17 (77.3)</td>
<td>997 (58.9)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Continuous variables (mean and 95% confidence interval)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>58.3 (55.9, 60.7)</td>
<td>60.5 (57.0, 63.9)</td>
<td>59.8 (56.2, 63.4)</td>
<td>65.1 (64.5, 65.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>59.8 (57.5, 62.2)</td>
<td>61.9 (58.5, 65.5)</td>
<td>61.1 (57.3, 64.9)</td>
<td>66.3 (65.8, 66.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Continuous variables (median and interquartile range)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival from onset (months)</td>
<td>28.0 (17.0, 44.0)</td>
<td>31.0 (20.0, 48.0)</td>
<td>41.0 (25.0, 59.0)</td>
<td>26.0 (16.0, 45.0)</td>
<td>0.45</td>
</tr>
<tr>
<td>Survival from diagnosis (months)</td>
<td>15.0 (8.0, 26.0)</td>
<td>17.0 (7.0, 26.0)</td>
<td>22.0 (17.0, 42.0)</td>
<td>14.0 (6.0, 28.0)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Motor site of onset: Classical FALS (n=64), SALS (n=1692). Significance testing performing using Chi-square, one-way ANOVA with Bonferroni correction and Kaplan Meier curve with log-rank analysis.

**Figure 9-1: Mean ages of onset and diagnosis by familial cohort**
9.5.1. Results: Impact of C9orf72 repeat expansion

DNA samples were available for 38 Classical FALS probands, 43 expanded FALS (with schizophrenia) probands, 19 expanded FALS (with FTD) probands and 624 sporadic ALS patients. All sporadic patients were negative for the pathogenic C9orf72 repeat expansion. 32/38 (84.2%) of those with classical FALS carried the repeat expansion. In contrast, the vast majority of both expanded familial ALS cohorts did not carry the repeat expansion (C9orf72 positive: expanded FALS [schizophrenia] 7/43 [16.3%], expanded FALS [FTD] 4/19 [21.1%]) (p=<0.001) (Figure 9-2).

Across all familial cohorts, there were no differences in mean ages of onset and diagnosis between C9orf72 carriers and non-carriers (age of onset: C9orf72 positive 57.8 years [95% CI 55.7, 59.9], negative 60.2 years [95% CI 56.9, 63.4], p=0.24; age of diagnosis: C9orf72 positive 58.8 years [95% CI 56.7, 60.0], negative 61.7 years [95% CI 58.3, 64.9], p=0.15). However, when those known to carry the repeat expansion were excluded from analysis, the expanded FALS (with schizophrenia) cohort were no longer significantly younger at onset than those with sporadic ALS (p=0.21) (Table 9-9). Those with classical FALS remained significantly younger at onset (p=0.023) and diagnosis (p=0.043) than those with sporadic ALS.

Figure 9-2: Proportion of familial ALS probands who carry the pathogenic C9orf72 repeat expansion

Classical FALS: C9orf72 positive (84.2%), negative (15.8%); Expanded FALS [schizophrenia]: C9orf72 positive (16.3%), negative (83.7%); Expanded FALS [FTD]: C9orf72 positive (21.1%), negative (78.9%);
Table 9.9: Comparison of mean ages of onset and diagnosis by familial status (C9orf72 carriers excluded)

<table>
<thead>
<tr>
<th></th>
<th>Classical FALS (n=32)</th>
<th>Expanded FALS (SCZ) (n=41)</th>
<th>Expanded FALS (FTD) (n=18)</th>
<th>SALS (n=1814)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset (years)</td>
<td>58.6 (54.1, 63.1)</td>
<td>61.2 (57.2, 65.1)</td>
<td>59.9 (55.5, 64.3)</td>
<td>65.1 (64.5, 65.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>60.7 (56.6, 64.8)</td>
<td>62.8 (58.8, 66.8)</td>
<td>61.3 (56.7, 65.9)</td>
<td>66.3 (65.8, 66.9)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

9.6. Discussion

In this study, I propose criteria by which to expand the current definition of familial ALS to encompass neuropsychiatric manifestations based on a novel probability modelling approach. I have shown clinical support for the importance of such an expansion in the observation of a younger mean age of ALS onset in probands from kindreds with clustering of FTD or schizophrenia. Application of two probability models was used to estimate the probability of occurrence of various degrees of familial clustering of disease, with both models generating congruent results. The probability of having one relative with FTD was found to exceed 5% only in kindreds of 35 persons or more. As the mean number of first- and second-degree relatives per ALS proband was 23 persons in the pooled family aggregation study (Chapter 8), this would suggest that the presence of one first- or second-degree relative with FTD is a credible criterion by which to extend the definition of familial ALS. By contrast, kindreds of 13 or greater may have at least one relative with schizophrenia by chance alone. The smaller kindred size may explain the apparent discrepancy in the Poisson model where one first-degree relative with schizophrenia is sufficient to define significant familial clustering, yet two or more relatives are required if second-degree relatives are also considered. In the same manner, as kindreds of 19 or more introduce a ≥5% risk of having a relative with ALS by chance alone, two or more first- or second-degree relatives with ALS are required to define familial ALS in the classical sense. Having one relative with ALS would only be considered significant in the presence of other relatives with FTD or schizophrenia.

Arguably more complex models of familial clustering could be built to truly encompass the exact probability of each relative developing a given disorder over their lifetime. Yet, while certainly interesting and worthwhile, that was not the aim of this study. Primarily, the objective of this endeavour was to build a simple model which would allow one to recognise what constitutes significant familial clustering of disorders in a busy clinical or
research setting. The Poisson model offers the advantage of providing a simple heuristic approach that does not require the history taker to determine the exact number of relatives in a given kindred. While the binomial approach of calculating the exact probability of observing the number of occurrences of specific disorders in a known kindred size which may be advantageous in certain contexts (e.g., small kindred size), in general, the former approach is preferable as it is simpler and more user-friendly to apply.

In this study, the clinical impact of the proposed novel expanded definitions of familial ALS encompassing associated neuropsychiatric disorders were explored. Patients with the more classical definition of familial ALS have previously been shown to develop the disease at an earlier age. This study found that this was also the case for the expanded familial ALS cases (those encompassing significant family histories of schizophrenia or FTD). Models of ALS development suggest earlier disease onset implies greater genetic risk in the individual. This hypothesis is supported by work by Mehta et al (2019) who showed that the younger age of onset in familial ALS resulted from pathogenic gene mutations, rather than ascertainment bias. In their study, those with familial ALS developed symptoms approximately 5 years earlier than those with sporadic forms of the disease, similar to the findings of this study. Interestingly, Mehta et al (2019) also noted that those with apparently sporadic ALS who carried a known ALS associated genetic mutation were also significantly younger at disease onset than "non-genetic" sporadic ALS cases. Earlier mean ages of onset have been reported in cohorts of SOD1, FUS and C9orf72 gene mutation carriers, providing clinical support to the findings of Chio et al (2018) that the presence of such mutations shorten the multistep process for ALS development. Cases of young-onset ALS patients carrying genetic mutations in SETX, SPG11, VAPB, ANG, ATXN2, UBQLN2 and SIGMAR1 have also been reported. However, none of these mutations were identified among the expanded familial ALS cohorts.

Indeed, the vast majority of families with ALS and schizophrenia were not explained by the C9orf72 repeat expansion. This aligns with findings by O'Brien et al (2017) that clustering of neuropsychiatric disorders within ALS kindreds, was not fully accounted for by the C9orf72 repeat expansion, with the authors suggesting that additional pleiotropic ALS genes may occur in the Irish context. While, this would appear to contradict the findings of the pooled family aggregation analysis (Chapter 8) which showed an increased risk of schizophrenia only among relatives of C9or72 positive probands, it is possible that the discrepancy in the finding may be attributable to differences in the study
designs employed. In the pooled family aggregation analysis, all relatives from C9orf72 negative kindreds were analysed together. This approach would mask the effect of a rare Mendelian-inherited variant, driving an increased risk of schizophrenia in a select number of kindreds. Probands from only 17 kindreds with significant familial clustering of ALS and schizophrenia were included in pooled family aggregation analysis, of which 15 did not carry the C9orf72 repeat expansion. In comparison, 44 C9orf72 positive ALS probands provided information on their relatives, as did a further 283 C9orf72 negative ALS probands (without family history of ALS or schizophrenia).

This is not to discount the possibility that C9orf72 repeat expansion carriers may present with psychotic symptoms. Indeed, Snowden et al (2012)\textsuperscript{203} reported that nearly 40\% of their C9orf72 positive FTD cohort presented initially with psychotic symptoms, although this clinic-based population may be biased somewhat towards atypical presentations. Furthermore, a smaller but similar family aggregation study by Devenney et al (2018)\textsuperscript{355} supported an increased risk of developing schizophrenia for relatives of C9orf72 carriers compared with noncarriers, highlighting the importance of the psychiatric phenotype within C9orf72 kindreds. It is interesting to note that once C9orf72 positive probands were excluded from analysis, probands from ALS kindreds with clustering of schizophrenia were no longer significantly younger than sporadic ALS patients. This most likely reflects reduced power for this analysis. It is interesting also to note that probands from classical familial ALS kindreds remained younger at onset than sporadic ALS cases, even after all C9orf72 positive probands were excluded. This supports the oligogenic hypothesis for ALS\textsuperscript{257}, which states that multiple genes are likely to contribute to the disease development. Similarly, and as has been observed with ALS and FTD\textsuperscript{137}, it is also likely that be other pleiotropic gene variants, beyond C9orf72, may contribute to the clustering of ALS and schizophrenia within certain kindreds.

\subsection*{9.6.1. Limitations}

While it is reassuring that both probability models used in this study were congruent in their findings, both models also have inherent limitations. Firstly, regarding the binomial distribution model, the lifetime risk data input was not in all cases specific to the Irish population (FTD, unspecified dementia). In addition, overall, rather than sex-specific lifetime risk estimates for ALS in the Irish population\textsuperscript{515} were used. As discussed above, more complex probability models may be built which account for kindred size and sex-specific risks. However, the preferred approach in this study was to build a simple heuristic model to allow for greater clinical utility.
Secondly, the Poisson distribution model relies on the average number of affected relatives with a given disorder reported by probands. This is not a true measure of the lifetime risk among relatives of probands, as feasibly some relatives (e.g., grandchildren) may develop a disorder decades after the proband first reported on their medical history. Yet for the purposes of family history taking with ALS probands, a grandchild who later develops a disorder, to a certain extent, is of little relevance to the proband in terms of recognising significant familial clustering. At such a time that this comes to pass, the proband may be long deceased. Instead, what is needed is a point in time measurement of how often similar patients report clustering of a given disease. The pooled family aggregation analysis (Chapter 8) provides an excellent source for these data having collected family history information on nearly 400 ALS probands over a ten-year time period. In this respect, the hidden variable to be cognisant of is the average kindred size reported by ALS probands. In the pooled analysis, the average number of first- and second-degree relatives reported on, by ALS probands, was approximately 23 persons. Should this change significantly over time, the definitions proposed above may need to be revised.

Finally, for the purposes of building a probability model, ALS and FTD were treated separately although it is acknowledged that both disorders may co-occur within one individual. The expanded criteria for significant clustering of ALS and FTD within kindreds proposed above does not account for individuals with ALS-FTD, as the probability models used to develop these criteria assume the disorders occur independently in separate individuals. As such, the significance of an ALS proband reporting of a relative with ALS-FTD has not yet been determined.

This limitation may in part explain the surprising absence of the C9orf72 repeat expansion among probands from the expanded ALS and FTD kindreds. The objective of the clinical comparative analysis in this study was primarily to determine the clinical relevance of having one or more relatives with FTD in the absence of an otherwise significant family history of ALS. This was to clarify if expanding the definition of familial ALS to encompass those with relatives with FTD only was worthwhile. Indeed, a younger mean age of onset in this cohort was observed, although the small sample size limited the power to detect a significant effect. In fact, significant clustering of ALS and FTD occurred in many kindreds, many of whom met criteria for classical familial ALS. The vast majority of probands from those kindreds carried the pathogenic repeat expansion. As such, it is clear that the C9orf72 repeat expansion is strongly associated with significant clustering of both ALS and FTD within kindreds. However, still this study also
highlights that other pathogenic mutations should be considered in kindreds with significant clustering of FTD, but not ALS.

9.6.2. Conclusion

The knowledge that the risk of developing neuropsychiatric disorders is increased among relatives of people with ALS, has resulted in the recent increased identification of ALS kindreds with clustering of these disorders. In this chapter, I used a novel probability modelling approach and applied data from Chapters 5 and 8, to delineate when such clustering within ALS kindreds exceeds that of a chance occurrence. I have shown that ALS probands from kindreds with significant clustering of schizophrenia or FTD have a younger age of disease onset, implying a higher shared genetic burden of risk exists within these families. Furthermore, I have identified that this increased genetic risk is not entirely attributable to the pathogenic C9orf72 repeat expansion, suggesting that other rare, pleiotropic gene variants are yet to be discovered in the Irish population. In this way, this study's work on delineating the importance of familial clustering of the neuropsychiatric signal has assisted in the identification and prioritisation of informative ALS kindreds for future genetic studies.
10. Chapter 10: Results Part VI: Comparison of Performance of Relatives of People with ALS and Healthy Controls on ECAS

10.1. Introduction

Chapters 10, 11 and 12 address Project Aim 3 ‘To characterise the sub-clinical phenotype of those at increased risk of developing ALS’. Each chapter discuss a separate sub-study which employ different assessment procedures to compare relatives of people with ALS against a healthy control population, with the objective of identifying novel cognitive endophenotypes in ALS. In each study, three levels of analyses are conducted relevant to the chapter sub-aims: 1) The performance of relatives on various measures is compared to that of the healthy control population; 2) If relatives demonstrate a greater degree of impairment, whether clustering of this signal occurs within familial kindreds is explored and 3) The impact of the pathogenic C9orf72 repeat expansion on “asymptomatic” relative performance is examined. This chapter pertains to data obtained using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), a brief ALS-specific cognitive screening tool.

10.2. Methods

Recruitment procedures, inclusion/exclusion and data collection processes for all ALS relative studies are outlined in Chapter 4. Familial ALS is defined in accordance to Byrne's criteria (2011)\textsuperscript{246}. Pathogenic C9orf72 repeat expansions are considered as expansions of greater than 30 repeats detected using repeat primed PCR. Results of continuous variables are reported as median and interquartile range and comparisons between groups for continuous variables are made using non-parametric tests (Mann Whitney U test [two groups] or Kruskal-Wallis test [greater than two groups]). Where data were normally distributed, independent sample T-tests or one way ANOVA were used instead to test significance.

To control for impact of age in the ECAS and neuropsychiatric trait studies, participants were sub-categorised into four groups based on their age at the time of assessment (<45, 45-54, 55-64 and 6+ years). For each age category, performance was compared across study groups using independent samples Kruskal Wallis test. In addition, Jonckheere-Terpstra tests were used on ECAS scores specifying a decreasing trend for cognition, and on neuropsychiatric trait scores specifying an increasing trend for psychiatric distress, to examine whether performance varied depending on the age category of the participant. Bonferroni corrections were applied to correct for multiple comparisons.
10.2.1. Participant recruitment

Between 1st January 2015 and 31st December 2017, 433 Irish incident ALS cases registered with the Irish ALS Register. Of these 147 patients completed a comprehensive neuropsychological assessment as a part of a longitudinal population-based research project examining cognitive and behavioural changes in people with ALS\textsuperscript{177}. Probands from these families were approached, where possible, to invite their relatives to participate in this research project. Participants could partake in any or all aspects of the project. Relatives from 31 families agreed to partake. 41 families declined to participate. In 54 cases, the proband died before contact was made and in 10 cases it was not possible to contact the proband or next of kin. 11 families were recruited for this study but due to the COVID-19 pandemic it was no longer possible to arrange home visits for this study. They will be offered the opportunity to participate in a follow-on study conducted with a minimal contact approach.

Concurrently, to enhance recruitment of relatives from familial ALS kindreds, all familial ALS kindreds with at least one proband diagnosed between 1st January 2008 and 31st December 2019 were identified (n=175 families). Of these, relatives from 34 kindreds agreed to participate in the research project. 42 families declined and 95 families were not contacted. Relatives from four families had expressed interest in partaking in the research, but this was halted due to the COVID-19 pandemic. As above, they will be offered the opportunity to participate in future familial ALS research studies.

In total, 56 unique families participated in the project, with an overlap of 10 families shared between both recruitment strategies. 231 relatives agreed to participate in different aspects of the project. 226 relatives donated a blood sample for genetic research. 214 relatives completed the ECAS cognitive screening test, with 147 of these completing the more detailed neuropsychological assessment study. Finally, 147 relatives complete an online neuropsychiatric traits assessment. A flow chart outlining the participant recruitment process is provided in Figure 10-1.
Figure 10-1: Flow chart of recruitment for ALS relatives studies

a. 10 families were shared between both recruitment strategies

Results

10.3. Demographics

ECAS data on 214 relatives (familial [157], sporadic [57]) were compared with that of 128 healthy controls. In the familial ALS relative cohort, 31 relatives carrying the pathogenic C9orf72 repeat expansion completed the assessment along with 58 non-carriers from the same kindreds. An additional 52 C9orf72 negative relatives from other familial ALS kindreds (not known to carry any pathogenic ALS mutation) took part in the
study. Finally, 52 C9orf72 negative relatives from sporadic ALS kindreds completed the assessment.

Relatives were younger and more educated than controls at the time of assessment (median age: relatives 42.0 years [IQR 32.0, 58.0], controls 62.0 years [IQR 55.0, 69.0], p<0.001; median years of education: relatives 16.0 years [IQR 14.0, 19.0], controls 13.8 years [IQR 11.0, 17.0], p<0.001). Education levels were similar for across relative cohorts (p=0.59). However, familial ALS relatives were younger than sporadic ALS relatives (median age: familial ALS relatives: 39.0 years [IQR 31.0, 55.0], sporadic ALS relatives 52.0 years [IQR 35.0, 63.5], p=0.023). There was no difference in the age profile of C9orf72 positive and negative relatives (median age: C9orf72 positive 38.0 years [IQR 33.0, 50.0], C9orf72 negative relatives 43.5 years [IQR 32.0, 59.0], p=0.42).

10.4. ECAS performance: Comparison relatives and controls

Relatives performed worse than controls on Total ECAS and ECAS ALS-specific scores (median ECAS total score: relatives 112.0 [IQR 103.0, 118.0], controls 115.0 [IQR 107.5, 121.0], p=0.002; median ECAS ALS-specific score: relatives 83.0 [IQR 75.0, 88.0], controls 86.0 [IQR 80.0, 90.0], p=0.001). Differences in ALS-specific scores arose from differences across language and verbal fluency scores (language p=0.005, verbal fluency p<0.001), with no difference observed on executive scores (p=0.18). No significant difference was detected across ECAS ALS non-specific sub-scores (relatives 29.0 [IQR 26.0, 31.0], controls 30.0 [IQR 27.0, 32.0], p=0.073).

10.5. ECAS performance: Comparison familial ALS relatives, sporadic ALS relatives and controls

Significant differences in ECAS total, ECAS ALS-specific and verbal fluency scores were evident across familial ALS relatives, sporadic ALS relatives and control cohorts (Table 10-1), driven by the poor performance in familial ALS relatives cohort. After controlling for multiple comparisons, familial ALS relatives performed worse than controls on ECAS total (p=0.001), ALS-specific (p=0.001), language scores (p=0.007) and verbal fluency scores (p=<0.001). Familial ALS relatives also performed worse than sporadic ALS relatives on verbal fluency tasks (p=0.045).

Table 10-2 compares performances on language and verbal fluency sub-tests across familial ALS relatives, sporadic ALS relatives and control cohorts. The poor verbal fluency performance in familial ALS relatives cohort is explained by the lower total number of words generated in each task. Familial ALS relatives generated less words than both sporadic ALS relatives and controls for unrestricted verbal fluency (p=0.003
and p<0.001 respectively) and less words than controls for the restricted paradigm (p <0.001). In the language sub-tests, familial ALS relatives also performed worse than controls on the spelling subtask (p=0.008). No differences between sporadic ALS relatives and controls were detected for any ECAS score.

Table 10-1: Comparison of ECAS performance across familial ALS relatives, sporadic ALS relatives and controls

<table>
<thead>
<tr>
<th></th>
<th>FALS relatives (n=157)</th>
<th>SALS relatives (n=57)</th>
<th>Controls (n=131)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAS total</td>
<td>110.0 (101.0, 118.0)</td>
<td>113.5 (107.0, 118.8)</td>
<td>115.0 (107.5, 121.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>ECAS ALS specific</td>
<td>83.0 (74.0, 87.0)</td>
<td>85.0 (78.3, 88.8)</td>
<td>86.0 (80.0, 90.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>ECAS ALS non-specific</td>
<td>29.0 (26.0, 31.0)</td>
<td>30.0 (27.0, 31.0)</td>
<td>30.0 (27.0, 32.0)</td>
<td>0.17</td>
</tr>
<tr>
<td>Language</td>
<td>27.0 (25.0, 28.0)</td>
<td>27.0 (26.0, 28.0)</td>
<td>27.0 (26.0, 28.0)</td>
<td>0.010</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>18.0 (16.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Executive</td>
<td>38.0 (33.3, 41.8)</td>
<td>39.0 (35.0, 42.0)</td>
<td>40.0 (35.0, 43.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Memory</td>
<td>18.0 (14.5, 20.0)</td>
<td>18.0 (16.0, 19.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

non parametric tests, median and IQR

Table 10-2: Comparison of performance on ECAS sub-tests (language and verbal fluency) across familial ALS relatives, sporadic ALS relatives and controls

<table>
<thead>
<tr>
<th></th>
<th>FALS relatives (n=157)</th>
<th>SALS relatives (n=57)</th>
<th>Controls (n=131)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal fluency (unrestricted)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total words</td>
<td>14.0 (10.0, 16.5)</td>
<td>16.0 (13.0, 19.0)</td>
<td>17.0 (13.0, 20.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to read words</td>
<td>7.3 (6.0, 10.0)</td>
<td>9.0 (8.0, 12.0)</td>
<td>11.5 (8.0, 16.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFI</td>
<td>3.9 (3.1, 5.4)</td>
<td>3.2 (2.4, 3.9)</td>
<td>2.8 (2.2, 3.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Verbal Fluency restricted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total words</td>
<td>7.0 (4.0, 10.0)</td>
<td>8.0 (6.0, 11.0)</td>
<td>10.0 (6.0, 12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to read words</td>
<td>3.7 (2.0, 6.0)</td>
<td>4.0 (3.0, 6.0)</td>
<td>7.0 (3.2, 9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VFI</td>
<td>8.1 (5.5, 14.5)</td>
<td>7.0 (4.9, 9.7)</td>
<td>5.6 (4.3, 9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Language</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naming</td>
<td>8.0 (7.0, 8.0)</td>
<td>8.0 (7.0, 8.0)</td>
<td>8.0 (7.0, 8.0)</td>
<td>0.27</td>
</tr>
<tr>
<td>Comprehension</td>
<td>8.0 (8.0, 8.0)</td>
<td>8.0 (8.0, 8.0)</td>
<td>8.0 (8.0, 8.0)</td>
<td>0.062</td>
</tr>
<tr>
<td>Spelling</td>
<td>11.0 (10.0, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

non parametric tests, median and IQR
10.6. ECAS performance: Comparison C9orf72 positive and negative ALS relatives and controls

ECAS data from relatives and control, by C9orf72 status and kindred, are compared in Table 10-3. ECAS total and ALS-specific scores were similar across all familial ALS relative cohorts, irrespective of C9orf72 status. C9orf72 positive ALS relatives (n=31), C9orf72 negative ALS relatives from these C9orf72-positive kindreds (n=58) and C9orf72 negative ALS relatives from other familial ALS kindreds (n=52) all performed worse than controls on ECAS total and ALS-specific scores. In particular, they performed worse than controls on measures of verbal fluency (C9orf72 positive ALS relatives [p=0.003], C9orf72 negative ALS relatives from C9orf72 kindreds [p<0.001], C9orf72 negative ALS relatives from other familial ALS kindred [p<0.001]) (Figure 10-2). No differences were detected between C9orf72 negative relatives from sporadic ALS kindreds and controls on any ECAS score.
Table 10-3: Comparison of ECAS performance across C9orf72 positive relatives, C9orf72 negative relatives and controls

<table>
<thead>
<tr>
<th></th>
<th>C9orf72 positive relatives (FALS♦) (n=31)</th>
<th>C9orf72 negative relatives (FALS♦) (n=58)</th>
<th>C9orf72 negative relatives (FALS♠) (n=52)</th>
<th>C9orf72 negative relatives (SALS) (n=52)</th>
<th>Controls (n=131)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAS total</td>
<td>110.0 (97.0, 119.0)</td>
<td>110.0 (101.0, 116.0)</td>
<td>111.0 (100.0, 121.0)</td>
<td>113.0 (107.0, 119.0)</td>
<td>115.0 (107.5, 121.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>ECAS ALS specific</td>
<td>84.0 (75.0, 88.0)</td>
<td>82.0 (73.0, 86.0)</td>
<td>82.0 (74.0, 88.0)</td>
<td>85.0 (78.0, 88.0)</td>
<td>86.0 (80.0, 90.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>ECAS ALS non-specific</td>
<td>29.0 (26.0, 31.0)</td>
<td>29.0 (25.0, 31.0)</td>
<td>30.0 (27.0, 32.0)</td>
<td>30.0 (27.3, 31.0)</td>
<td>30.0 (27.0, 32.0)</td>
<td>0.31</td>
</tr>
<tr>
<td>Language</td>
<td>26.0 (24.0, 28.0)</td>
<td>27.0 (25.0, 27.0)</td>
<td>27.0 (25.0, 28.0)</td>
<td>27.0 (26.0, 28.0)</td>
<td>27.0 (26.0, 28.0)</td>
<td>0.013</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>18.0 (16.0, 20.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Executive</td>
<td>38.0 (33.3, 41.8)</td>
<td>38.0 (33.5, 41.0)</td>
<td>38.0 (32.3, 42.0)</td>
<td>39.0 (35.0, 42.0)</td>
<td>40.0 (35.0, 43.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>Memory</td>
<td>18.0 (14.5, 20.0)</td>
<td>18.0 (14.0, 19.3)</td>
<td>18.0 (16.0, 20.0)</td>
<td>18.0 (16.0, 19.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>12.0 (11.0, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>12.0 (12.0, 12.0)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

non-parametric, median IQR. ♦ C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♠ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.
10.7. Impact of age

10.7.1. Demographics

To examine the relationship between age, education and cognitive performance, participants were sub-categorised into four groups based on their age at the time of assessment (<45, 45-54, 55-64 and 64+ years). For each age category, differences in median ECAS total, ALS-specific, ALS non-specific and sub-categories scores, as well as numbers of years of education were compared across familial ALS relatives, sporadic ALS relatives and controls groups. Jonckheere-Terpstra tests were used for each study group to examine whether cognitive performance varied depending on the age category of the participant.

Among participants under the age of 45 years, there were 95 familial ALS relatives, 22 sporadic ALS relatives and 3 controls. Included in the 45–54-year-old age category were 21 familial ALS relatives, 8 sporadic ALS relatives and 27 controls. There were 21 familial ALS relatives, 13 sporadic ALS relatives and 45 controls in the 55–64-year-old category. Finally, 18 familial ALS relatives, 13 sporadic ALS relatives and 54 controls were aged 65 years or more at the time of assessment. For each age category, there were no
differences across groups in years of education (< 45 years [p=0.31], 45-54 [p=0.15] and 65+ [p=0.20]), except for the 55–64-year-old category in which sporadic ALS relatives were found to be more highly educated than both familial ALS relatives (p=0.012) and controls (p=0.027).

10.7.2. **ECAS performance: Comparison of familial and sporadic ALS relatives and control cohorts within separate age categories**

10.7.2.1. **ECAS total and ALS-specific**

A comparison of ECAS scores across familial and sporadic ALS relatives and controls per age category is made in Table 10-4. For those aged 45 years or older, ECAS total and ALS-specific scores differed significantly across familial ALS relatives, sporadic ALS relatives and control groups. In all cases, the differences across groups were determined by significant performance differences between familial ALS relatives and controls. ECAS total score was also close to significantly different between familial and sporadic ALS relatives among those aged 65 years or older (p=0.058).

The ECAS ALS-specific sub-scores of language, verbal fluency and executive function were examined to determine in which domain the intergroup differences arose. In the 45–54-year-old category, significant differences between familial ALS relatives and controls were detected for the executive domain (p=0.039), with borderline differences detected for verbal fluency (p=0.051) and language (p=0.064). In the same age category, sporadic ALS relatives differed significantly from controls on language tasks (p=0.019). In the 55–64-year-old category, familial ALS relatives differed significantly from controls for both verbal fluency (p=0.039) and language (p=0.027), but not executive function. In those aged 65 years or older, the greatest differences were detected across familial ALS relatives and control groups for verbal fluency (p<0.001), with differences also detected in this domain between familial and sporadic ALS relatives (p=0.011). Familial ALS relatives also differed from controls on both language (p=0.031) and executive (p=0.033) tasks.

10.7.2.2. **ECAS ALS non-specific**

Examining ECAS ALS non-specific scores, significant differences across groups were only detected in those aged 65 years or older, again driven by differences between familial ALS relatives and controls (p=0.012). In turn, this can be attributed to differences between the groups on memory tasks in this age group (p=0.034).
10.7.3. **ECAS performance: Comparison of familial and sporadic ALS relatives and control cohorts within separate age categories**

The results of Jonckheere-Terpstra tests examining if ECAS total and sub-score performances changed across age categories are reported in Table 10-5. For controls and sporadic ALS relatives, no differences in cognitive performance were evident. In contrast, the cognitive performance of familial ALS relatives on ECAS declined with increasing age category. Visual inspection of raw scores evidenced a steady decline in ECAS total and ALS-specific scores among familial ALS relatives after the age of 45, with ALS non-specific scores declining in those over 55 years of age (Figure 10-3). However, significant differences on ECAS total and sub-score performance were only detected between the youngest (<45 years) and oldest (65+years) age categories, after correction for multiple comparisons.
Table 10-4: Comparison of ECAS performance scores across familial ALS relatives, sporadic ALS relatives and control cohorts by age category

<table>
<thead>
<tr>
<th></th>
<th>Age category</th>
<th>&lt;45 years</th>
<th>45-54 years</th>
<th>55-64 years</th>
<th>65+ years</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAS total</td>
<td>Controls</td>
<td>114.0 (100.0, 118.0)</td>
<td>117.0 (106.3, 123.0)</td>
<td>115.5 (111.3, 119.8)</td>
<td>114.0 (106.8, 121.3)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>115.0 (108.0, 122.0)</td>
<td>110.0 (107.3, 115.0)</td>
<td>118.0 (109.5, 120.5)</td>
<td>113.0 (102.5, 118.0)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>114.0 (106.0, 120.0)</td>
<td>107.0 (100.0, 114.0)</td>
<td>104.0 (93.5, 119.0)</td>
<td>98.5 (91.3, 109.0)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.80</td>
<td>0.019</td>
<td>0.020</td>
<td>0.002</td>
</tr>
<tr>
<td>ECAS ALS specific</td>
<td>Controls</td>
<td>84.0 (76.0, 90.5)</td>
<td>86.5 (78.5, 92.0)</td>
<td>87.0 (81.5, 90.0)</td>
<td>84.0 (78.8, 90.0)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>86.5 (77.0, 92.0)</td>
<td>80.5 (78.0, 87.0)</td>
<td>86.0 (81.0, 88.0)</td>
<td>85.0 (73.5, 87.5)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>84.0 (78.0, 88.0)</td>
<td>79.0 (71.5, 85.5)</td>
<td>75.0 (65.0, 87.5)</td>
<td>75.5 (63.5, 82.0)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.75</td>
<td>0.015</td>
<td>0.017</td>
<td>0.003</td>
</tr>
<tr>
<td>ECAS ALS non-specific</td>
<td>Controls</td>
<td>30.0 (24.0, 34.8)</td>
<td>30.5 (28.0, 32.5)</td>
<td>29.0 (27.0, 32.0)</td>
<td>30.0 (27.0, 32.0)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>30.0 (28.0, 31.0)</td>
<td>27.5 (26.3, 31.5)</td>
<td>30.0 (28.0, 31.5)</td>
<td>29.0 (26.5, 31.0)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>30.0 (27.0, 32.0)</td>
<td>30.0 (24.5, 32.5)</td>
<td>28.0 (24.5, 30.0)</td>
<td>27.0 (22.0, 30.0)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.73</td>
<td>0.41</td>
<td>0.14</td>
<td>0.015</td>
</tr>
<tr>
<td>Language</td>
<td>Controls</td>
<td>26.0 (24.0, 28.0)</td>
<td>28.0 (27.0, 28.0)</td>
<td>27.0 (26.0, 28.0)</td>
<td>27.0 (25.0, 28.0)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>27.0 (25.8, 28.0)</td>
<td>26.5 (25.3, 27.0)</td>
<td>27.0 (26.5, 27.5)</td>
<td>27.0 (26.5, 27.5)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>27.0 (25.0, 28.0)</td>
<td>27.0 (25.0, 27.5)</td>
<td>26.0 (23.5, 27.0)</td>
<td>26.0 (23.0, 27.0)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.40</td>
<td>0.007</td>
<td>0.032</td>
<td>0.029</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Controls</td>
<td>20.0 (12.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>20.0 (20.0, 20.0)</td>
<td>20.0 (19.5, 20.0)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>20.0 (16.0, 20.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>20.0 (18.0, 20.0)</td>
<td>20.0 (19.0, 20.0)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>18.0 (18.0, 20.0)</td>
<td>18.0 (16.0, 20.0)</td>
<td>18.0 (10.0, 20.0)</td>
<td>16.0 (10.0, 20.0)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.52</td>
<td>0.039</td>
<td>0.043</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Executive</td>
<td>Controls</td>
<td>38.0 (37.0, 39.0)</td>
<td>41.5 (36.3, 44.4)</td>
<td>40.0 (35.5, 42.5)</td>
<td>38.0 (33.0, 42.0)</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>40.0 (34.0, 44.0)</td>
<td>37.5 (35.5, 41.0)</td>
<td>40.0 (37.5, 41.0)</td>
<td>39.0 (26.0, 41.0)</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>40.0 (36.0, 42.0)</td>
<td>36.0 (30.5, 40.0)</td>
<td>37.0 (25.5, 41.5)</td>
<td>35.0 (28.0, 37.3)</td>
</tr>
</tbody>
</table>
Figure 10-3: Comparison of ECAS performance scores across familial ALS relatives, sporadic ALS relatives and control cohorts, by age category.
Table 10-5: Jonckheere-Terpstra tests of ECAS performance across increasing age categories (<45 years, 45-54 years, 55-64 years and 65 years or more)

<table>
<thead>
<tr>
<th>ECAS score</th>
<th>Group</th>
<th>T_{JT}</th>
<th>p -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAS total</td>
<td>Controls</td>
<td>2507.0</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>501.5</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2241.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECAS ALS specific</td>
<td>Controls</td>
<td>2542.5</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>507.0</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2197.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECAS ALS non-specific</td>
<td>Controls</td>
<td>2765.0</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>548.0</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2729.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Language</td>
<td>Controls</td>
<td>2499.5</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>543.5</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2689.5</td>
<td>0.004</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Controls</td>
<td>2752.5</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>554.0</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2656.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Executive</td>
<td>Controls</td>
<td>2446.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>484.5</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2218.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Memory</td>
<td>Controls</td>
<td>2815.5</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>533.5</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2821.5</td>
<td>0.011</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>Controls</td>
<td>2503.5</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td>SALS relatives</td>
<td>583.0</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>FALS relatives</td>
<td>2761.5</td>
<td>0.14</td>
</tr>
</tbody>
</table>
10.8. C9orf72 positive relatives: Impact of age on cognitive performance

31 presymptomatic C9orf72 carriers completed ECAS testing (<45 years [21], 45-54 [3], 55-64 [6] and 65+ years [1]). Due to the small numbers, it was not possible to compare ECAS scores of C9orf72 positive relatives with those of C9orf72 negative relatives within each age category. However, Jonckheere-Terpstra tests supported a decline in cognitive performance across increasing age categories (ECAS total [TJT 48.0, p=0.003], ECAS ALS-specific [TJT 51.5, p=0.005], language [TJT 48.5, p=0.003], fluency [TJT 40.0, p=0.001] and executive function [TJT 69.0, p=0.040]).

Visual inspection of the raw ECAS scores of C9orf72 positive relatives (Figure 10-4), suggest similar cognitive performance between those aged less than 45 year and those aged between 45-54 years, with a significant drop off in performance for those aged between 55-64 years. Verbal fluency appeared to decline steadily across age groups. However, differences in cognitive performance on ECAS total, ALS-specific and sub-scores were only significant between the less than 45 years and 55–64-year-old age categories after correcting for multiple comparisons.

Applying Irish normative cut-off scores for ECAS total scores, 19/21 (90.5%) of C9orf72 positive relatives aged less than 45 were deemed cognitively normal, yet only 1/6 (16.7%) of those age between 55-64 years remained cognitively normal. This pattern was replicated for ECAS ALS-specific scores. 20/21 (95.2%) of those aged less than 45 scored in the normal range, while 1/6 (16.7%) of those aged 55-64 were abnormal. In contrast, for the ECAS ALS non-specific scores, the vast majority of both age categories were normal (20/21 [95.2%] and 5/6 [83.3%] respectively). One C9orf72 positive individual over 65 years of ages completed the assessment. While they scored in the normal range on ECAS total score, it is notable that they were categorised as abnormal on verbal fluency measures.
Comparison of ECAS performance across age categories was made for 164 C9orf72 negative relatives. A decrease in cognitive performance across increasing age categories was observed. This decline across age categories was driven only by C9orf72 negative relatives from C9orf72 kindreds (<45 [38], 45-54 [6], 55-64 [7] and 65+ years [7]). In C9orf72 negative relatives from other familial ALS kindreds and from sporadic ALS kindreds, no attenuation in performance for any ECAS measure was seen across age categories.

C9orf72 negative relatives from C9orf72 kindreds declined on ECAS total ($T_{JT}$ 193.0, $p<0.000$) and both ALS-specific ($T_{JT}$ 230.0, $p=0.002$) and non-specific sub-scores ($T_{JT}$ 227.5, $p<0.001$). The former driven by reducing performance on language ($T_{JT}$ 315.5,
p=0.033) and executive function tasks ($T_{JT}$ 236.5, $p=0.002$) and the latter by declining memory scores ($T_{JT}$ 227.5, $p<0.000$). No differences across age categories were seen for verbal fluency ($T_{JT}$ 381.5 [$p=0.28$]). On visual inspection, no clear drop off on performance across any age group was observed (Figure 10-5) and differences in ECAS scores were only significant between those aged less than 45 and those 65 years or older.

In contrast to the C9orf72 positive relatives, the vast majority of C9orf72 negative relatives from the same kindreds remained within normal cognitive cut-offs, irrespective of their age category (ECAS total score normal: less than 45 years [35/38 (92.1%)]; 65 years or older [6/6 (100.0%)]).

<table>
<thead>
<tr>
<th>Age Category</th>
<th>&lt;45 years</th>
<th>45-54 years</th>
<th>55-64 years</th>
<th>65+ years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECAS Total</strong></td>
<td>114.0 (107.0, 117.3)</td>
<td>105.0 (94.8, 111.5)</td>
<td>101.0 (97.0, 108.0)</td>
<td>93.5 (87.3, 107.8)</td>
</tr>
<tr>
<td><strong>ECAS ALS-Specific</strong></td>
<td>84.5 (79.0, 87.0)</td>
<td>76.0 (70.3, 85.0)</td>
<td>74.0 (70.0, 84.0)</td>
<td>69.0 (61.3, 83.5)</td>
</tr>
<tr>
<td><strong>ECAS ALS Non-specific</strong></td>
<td>30.5 (27.8, 32.0)</td>
<td>28.0 (23.8, 30.3)</td>
<td>28.0 (25.0, 30.0)</td>
<td>23.0 (21.0, 28.0)</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td>27.0 (25.0, 28.0)</td>
<td>26.5 (24.0, 27.3)</td>
<td>26.0 (25.0, 27.0)</td>
<td>24.0 (20.0, 27.0)</td>
</tr>
</tbody>
</table>
### Verbal fluency

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Mean (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45 years</td>
<td>18.0 (16.0, 20.0)</td>
</tr>
<tr>
<td>45-54 years</td>
<td>19.0 (16.0, 20.0)</td>
</tr>
<tr>
<td>55-64 years</td>
<td>20.0 (12.0, 20.0)</td>
</tr>
<tr>
<td>65+ years</td>
<td>14.0 (8.0, 20.0)</td>
</tr>
</tbody>
</table>

### Executive

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Mean (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45 years</td>
<td>39.0 (36.0, 41.3)</td>
</tr>
<tr>
<td>45-54 years</td>
<td>34.0 (29.0, 38.3)</td>
</tr>
<tr>
<td>55-64 years</td>
<td>37.0 (27.0, 38.0)</td>
</tr>
<tr>
<td>65+ years</td>
<td>34.0 (29.3, 36.3)</td>
</tr>
</tbody>
</table>

### Memory

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Mean (Lower, Upper)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45 years</td>
<td>19.0 (15.8, 20.0)</td>
</tr>
<tr>
<td>45-54 years</td>
<td>17.5 (11.8, 18.3)</td>
</tr>
<tr>
<td>55-64 years</td>
<td>17.0 (14.0, 18.0)</td>
</tr>
<tr>
<td>65+ years</td>
<td>11.0 (11.0, 17.0)</td>
</tr>
</tbody>
</table>

Figure 10.5: ECAS performance of C9orf72 negative relatives from C9orf72 kindreds, by age category (<45 years, 45-54 years, 55-64 years and 65+ years)

### 10.10. Summary of findings

A summary of the key findings from this section exploring the performance of ALS relatives and healthy controls on ECAS cognitive screening tool are outlined below.

1. Relatives of ALS patients demonstrated a unique profile of cognitive deficits, distinct from healthy controls, despite being younger and more highly educated. The pattern was consistent with that observed among ALS patients, with evident language and verbal fluency deficits and some executive and memory impairment.

2. The findings were primarily detected among familial ALS relatives, with sporadic ALS relatives and controls performing similarly on cognitive testing. Among those aged over 45 years, familial ALS relatives performed worse than controls on ECAS total and ALS-specific sub-scores. Familial ALS relatives aged 65 years or older, also performed worse than controls on ALS non-specific tasks, attributable to poor performance on memory tasks. The cognitive performance of familial ALS relatives on ECAS screening tool declined with increasing age category. This was not seen for sporadic ALS relatives or controls.

3. Assessment of C9orf72 positive relatives was limited by small numbers. Nonetheless, a decline in cognitive performance with increasing age categories was observed for ECAS total, ALS-specific, language, verbal fluency and executive function scores. This was further evidenced by the observation that over 90% of C9orf72 carriers less than 45 years old were cognitively normal using ECAS total score normality cut-offs. In contrast, only 17% of those aged 55-64 scored within normal parameters.

4. C9orf72 negative relatives from all familial ALS kindreds performed worse than controls on ECAS total and ALS specific scores, due to poor performance by both groups on verbal fluency tasks. However, only C9orf72 negative relatives from
C9orf72 kindreds showed evidence of declining performance across increasing age categories. Similar to C9orf72 positive relatives from the same kindreds, declining performance was noted for ECAS total, ALS specific, language and executive domains. Yet, in contrast to the C9orf72 positive relatives, differences were also detected on ALS non-specific and memory scores and C9orf72 negative relatives did not decline on verbal fluency tasks. Finally, despite the decline, the vast majority C9orf72 negative relatives from C9orf72 kindreds remained within normal cognitive cut-offs, irrespective of their age.

10.11. Discussion

This large study highlights the potential utility of the ECAS as a brief and highly sensitive screening tool for detecting cognitive changes, not only among people with ALS, but also in their asymptomatic relatives. Despite the protective effects of youth and education, relatives of people with ALS demonstrated widespread impaired performance across multiple cognitive domains, inferring that the genetic burden linked to ALS may have a substantial cognitive pre-morbid load. This is not unsurprising given that nearly half of ALS patients have some degree of cognitive impairment at diagnosis, necessitating either the existence of either a gradual pre-morbid decline or low baseline general cognitive function. Work by Bandres-Ciga et al (2019) would support the latter argument. These authors identified through interrogation of GWAS data that ALS polygenic risk is negatively correlated with higher cognitive abilities, supporting the ongoing search for cognitive endophenotypes in ALS as a means to identify novel ALS-associated gene variants.

Work in this field, to date, has focused nearly exclusively on the pre-symptomatic characteristics of known pathogenic ALS mutations. Owing to the rarity of some of these mutations, this work has often been conducted through large multi-site collaborations. Yet, in many instances, the research focus of these groups has prioritised biomarker identification (e.g., neuroimaging) over deep-phenotyping. Over-controlled study inclusion criteria (e.g., mutation carrier must be cognitively normal on extensive neuropsychological assessment) has rendered the true cognitive profiling of the ALS ‘genetically at risk’ population relatively understudied.

While the recruitment strategy in this study was loaded to recruit more relatives of familial ALS patients, the number of C9orf72 positive recruits remained low. Those C9orf72 positive relatives who participated in the project were predominantly younger, with the vast majority being under 45 years of age at the time of assessment. Despite these
limitations, declining cognitive performance on ECAS testing across increasing age categories was observed for ALS-specific domains, with the vast majority of those carriers aged between 55-64 years categorised as cognitively abnormal. This is likely to exacerbate difficulties with recruiting older C9orf72 positive relatives. These participants would be less likely to meet some studies’ inclusion criteria. Yet, arguably, these are the individuals who may be most informative in aiding our understanding of the biological processes at play and how they manifest clinically.

C9orf72 positive relatives in this study demonstrated deficits on verbal fluency measures. These have previously been reported by Lulé et al (2020)\textsuperscript{379} as being a distinct clinical feature of asymptomatic expansion carriers. Lulé et al (2020)\textsuperscript{379} also reported that performance on this measure did not change among carriers over a one-year follow-up period, supporting temporal stability for the task. However, this study suggests that that follow period may simply have been insufficient to detect meaningful clinical change. While visual examination of the study data suggests a drop-off in cognitive performance among C9orf72 carriers in their fifties, significant differences were only detected between the youngest and oldest age categories, the participants of which differ in age by at least 20 years. As such, the detection of meaningful differences in the “asymptomatic” C9orf72 positive cohort would require much longer follow up periods or the examination of an older cohort of mutation carriers.

The central novel finding of these studies however is the extent of cognitive changes observed among C9orf72 negative relatives from familial ALS kindreds. In particular, these relatives also performed poorly on verbal fluency measures, with the extent of deficit observed similar to that of C9orf72 carriers. This impairment was not confined to relatives from the C9orf72 kindreds, but rather was evident across all relatives from familial ALS kindreds. Verbal fluency difficulties are the most consistent deficit observed in patients with ALS and are thought to primarily stem from executive dysfunction, rather than represent primary linguistic difficulties\textsuperscript{440}. While work is required to assess the degree of correlation between verbal fluency performance among the relatives and probands from their kindreds, the familial clustering of impaired verbal fluency performance would mark verbal fluency as a potential cognitive endophenotype for ALS.

Although endophenotypes have been used in the field of psychiatric research for over four decades\textsuperscript{532}, only recently has there been a surge of interest surrounding the concept in neurology. As with work by the Consortium on the Genetics of Schizophrenia (COGS) which has identified several genes (CTNNA2, ERBB4, GRID2, GRIK3, GRIK4, NOS1AP, NRG1, and RELN) with pleiotropic associations across multiple psychiatric
endophenotypes, it is hoped that identifying cognitive endophenotypes in ALS will provide insights into shared genetic liability impinging on multiple extra-motor neural systems. The clustering of verbal deficits in familial ALS kindreds are clinical manifestations of a shared genetic risk with ALS kindreds, which is not fully explained by the C9orf72 repeat expansion. Considering ALS as an oligogenic disorder, this could suggest that other as-of-yet unidentified ALS associated genetic mutations occur within these ALS kindreds. The C9orf72 repeat expansion could interact with this risk, driving the disease process forward explaining the subsequent decline in verbal fluency in the cohort of C9orf72 carriers.

10.11.1. Limitations

The cognitive deficits detected among asymptomatic relatives were subtle, particularly among C9orf72 negative relatives, of whom, the vast majority tested within normal cognitive parameters across all age cohorts. While the ECAS is a highly sensitive screening tool, the findings of this study require verification with more detailed neuropsychological assessment. Normative data is also available for most commonly used neuropsychological tests, allowing for age-standardisation of data which would control in part for the potential impact of different age profiles across relatives and controls cohorts. However, this would not control for the impact of different environmental exposures across different age categories. For example, in this study, education levels differed between the older relatives and younger control cohorts. As education may play a protective role in offsetting cognitive impairment, the higher education levels of relatives compared with controls would render the degree of cognitive deficits detected in this study as potentially conservative estimates. Furthermore, the apparent decline across increasing age cohorts for C9orf72 carriers could be explained by the higher educational attainment in the younger cohort, although absence of a decline in non-C9orf72 kindred relatives and controls would argue against this. A longitudinal study with age and education-matched controls is required to fully elucidate the nature of this gene-environment interaction.

10.11.2. Conclusion

Cognitive impairment is increasingly recognised as a core feature of ALS, impacting a sub-set of patients. This chapter has demonstrated that relatives of people with ALS show widespread impairments across multiple cognitive domains, mirroring patterns seen in ALS patients. The clustering of these deficits in familial ALS kindreds highlights potential selective extra-motor networks vulnerabilities reflecting the increased genetic liability. Further work is required to investigate whether such deficits could serve as
worthwhile cognitive endophenotypes in ALS. Nonetheless, this current work would raise concerns as to the current common practice of using asymptomatic non-carriers as the only controls in studies of pre-symptomatic mutation carriers. This work highlights how such study design negate on the opportunity to advance our understanding of how oligogenic inheritance patterns impact on phenotypic manifestations in ALS.
11. Chapter 11: Results Part VII: Comparison of Performance of Relatives of People with ALS and Healthy Controls on Comprehensive Neuropsychological Assessment

11.1. Introduction

The previous chapter used the ECAS cognitive screening tool to explore the presence and severity of cognitive deficits among relatives of patients with ALS. In this chapter, the sub-clinical phenotype of those at increased risk of developing ALS is characterised further (Project Aim 3) using a comprehensive neuropsychological assessment. Study methodology procedures, including details of the full neuropsychological battery used, are outlined in Chapters 4 and 10 respectively. The results of this study are reported below.

Results

11.2. Demographics

149 ALS relatives (familial [96], sporadic [53]) and 58 controls completed the full neuropsychological assessment. In the familial ALS relative cohort, 11 relatives carrying the pathogenic C9orf72 repeat expansion completed the assessment along with 25 non-carriers from the same kindreds. An additional 47 C9orf72 negative relatives from other familial ALS kindreds (not known to carry any pathogenic ALS mutation) took part in the study. Finally, 51 C9orf72 negative relatives from sporadic ALS kindreds completed the assessment.

Relatives who completed the assessment were sex and education matched with controls (sex [p=0.82], education [p=0.44]). No differences between groups in current psychological distress at the time of assessment were detected (HADS [p=0.08], GHQ [p=0.69]). However, relatives were significantly younger at time of assessment than controls (median age controls 65.0 years [IQR 57.0, 72.8, p<0.001]). All neuropsychological data below are standardised to control for age.

11.2.1. Participants and non-participants

All relatives, who completed the ECAS study, were offered the opportunity to complete the full neuropsychological assessment. Relatives who did not participate in the more detailed assessment were less educated (p=0.002) and scored lower on ECAS ALS non-specific tasks (p=0.030) than those who did (Table 11-1). A greater proportion of non-
participants were familial ALS relatives (p<0.001) and over 30% of non-participants carried the C9orf72 repeat expansion (p<0.001).

Table 11-1: Comparison of participants and non-participants for full neuropsychological assessment

<table>
<thead>
<tr>
<th>Demographics (number, %)</th>
<th>Completed assessment (n=149)</th>
<th>Did not complete assessment (n=64)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>67 (45.0)</td>
<td>27 (42.2)</td>
<td>0.71</td>
</tr>
<tr>
<td>C9orf72 (positive)</td>
<td>11/141 (7.8)</td>
<td>20/62 (32.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FALS relative (yes)</td>
<td>96 (64.4)</td>
<td>61 (95.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.0 (31.0, 59.0)</td>
<td>37.0 (33.0, 55.8)</td>
<td>0.28</td>
</tr>
<tr>
<td>Education (years)</td>
<td>17.0 (15.0, 19.0)</td>
<td>14.1 (12.0, 17.0)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Performance on ECAS cognitive screen (median, IQR)

| ECAS total               | 112.0 (104.0, 119.0)         | 110.0 (100.0, 117.0)              | 0.23   |
| ECAS ALS specific        | 84.0 (76.0, 88.0)            | 82.0 (75.0, 88.0)                 | 0.54   |
| ECAS ALS non-specific    | 30.0 (27.0, 31.0)            | 28.0 (25.0, 31.0)                 | 0.030  |
| Language                 | 27.0 (26.0, 28.0)            | 26.0 (24.0, 27.0)                 | 0.049  |
| Verbal fluency           | 18.0 (16.0, 20.0)            | 18.0 (16.0, 20.0)                 | 0.89   |
| Executive                | 39.0 (34.0, 42.0)            | 38.0 (34.0, 42.0)                 | 0.58   |
| Memory                   | 18.0 (15.0, 20.0)            | 17.0 (14.0, 19.0)                 | 0.063  |
| Visuospatial             | 12.0 (11.0, 12.0)            | 12.0 (11.0, 12.0)                 | 0.97   |

Lower score on ECAS indicate worse performance.

11.3. Comparison of neuropsychological performance of relatives of people with ALS and controls

The results of the comparative analysis of age-standardised neuropsychological performance of relatives versus controls are reported in Table 11-2. The relatives performed worse than controls across multiple cognitive domains including executive function, language, memory and visuospatial abilities. In all cases, the between group differences detected were subtle (<1 standardised deviation).

In the executive function domain, relatives performed worse on standardised tests of verbal fluency, inhibition and working memory. Verbal fluency deficits were detected in relatives for unrestricted FAS total score (p<0.001), but not restricted fluency or semantic paradigms. Relatives made more inhibition errors than controls on the colour word interference task (p=0.001), but no significant difference was observed between groups for the switching/inhibition task. Relatives also performed poorly compared with controls on the digit span backwards task (p<0.001) which challenges both working memory and inhibitory control. No differences in performances were detected between groups for digit span forward or sequential tasks. On the SART task, no differences between groups
were detected for the number of omission, commission or anticipation errors. However, relatives had a faster mean reaction time than controls (p=0.014). Iowa gambling task results are reported as standardized summative total scores and scores for each of 5 sections which allows one to examine how participants modify their decisions over time in response to the stimuli. Relatives performed worse than controls on IGT total scores (p=0.035), driven by worse performance on the latter stages of the test (net 4 [p=0.036], net 5 [p=0.006]). This suggests that the relatives, unlike the controls, did not learn to avoid the disadvantageous stimuli over time. Figure 11-1 A.

With respect to memory tasks, relatives performed worse than controls on verbal recall (immediate: RAVLT total trials 1-5 [p=0.001]; delayed: RAVLT trial 7 [p=0.001]), episodic memory (logical memory delayed recall [p <0.001]) and visuospatial memory (RCFT: immediate [p<0.001], recognition [p=0.007]). The latter difference is not attributable to visuospatial deficits, as relatives and controls performed equally well in copying the figure (p=0.84), although relatives took longer to complete the task (p=0.008). Interestingly, on the RAVLT task, the relatives’ performance was worse than controls for all trials. The rate of learning over time was comparable for both groups, suggesting the differences in performance were attributable to worse initial recall among relatives (Figure 11-1 B).

Finally, in the language domain, relatives performed worse than controls on the Boston Naming test, for both spontaneous (p<0.001) and cued (p<0.001) responses. For both current and pre-morbid intellectual functioning, relatives performed worse than controls (WASI-II FSIQ [p=0.006], TOPF FSIQ [p=0.008]). While the latter differences may be attributable in part to differences in the age profile between the two cohorts, it is notable that these differences were replicated in the WASI-II FSIQ, including for both sub-scores (vocabulary [p=0.039], matrix reasoning [p=0.014]).

Table 11-2: Comparison of standardised neuropsychological performance of relatives of people with ALS and controls

<table>
<thead>
<tr>
<th></th>
<th>Relatives (n=149)</th>
<th>Controls (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current/pre-morbid intellectual functioning</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOPF FSIQ</td>
<td>103.6 (96.5, 113.0)</td>
<td>109.3 (100.1, 121.2)</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>WASI-II FSIQ*</td>
<td>100.5 (89.3, 100.7)</td>
<td>105.5 (89.8, 115.0)</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td><strong>Executive functioning</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>FAS total Z</td>
<td>-0.5 (-1.1, 0.4)</td>
<td>0.14 (-0.5, 1.2)</td>
</tr>
<tr>
<td>Animals total Z*</td>
<td>-0.2 (-0.7, 0.6)</td>
<td>-0.2 (-0.8, 0.7)</td>
<td>0.53</td>
</tr>
<tr>
<td>Colour-word interference test</td>
<td>Inhibition errors Z</td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.7 (0.4, 0.8)</td>
</tr>
<tr>
<td></td>
<td>Inhibition time Z</td>
<td>0.0 (-0.7, 0.7)</td>
<td>0.4 (0.0, 0.7)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Z</td>
<td>p-Value</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Switching errors</strong></td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Switching time</strong></td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.4 (-0.4, 1.0)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Digit span</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Forward Z</strong></td>
<td>0.1 (-0.6, 0.9)</td>
<td>0.4 (-0.4, 1.2)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Backwards Z</strong></td>
<td>0.1 (-0.6, 0.7)</td>
<td>0.5 (0.0, 1.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Sequential Z</strong></td>
<td>0.3 (-0.1, 0.8)</td>
<td>0.4 (0.1, 1.0)</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>SART</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Omission errors Z</strong></td>
<td>-0.0 (-1.0, 0.7)</td>
<td>0.4 (-0.2, 0.8)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Commission errors Z</strong></td>
<td>-0.1 (-1.0, 0.5)</td>
<td>0.2 (-0.5, 0.6)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Anticipation errors Z</strong></td>
<td>0.2 (-0.7, 0.5)</td>
<td>0.4 (-0.1, 0.4)</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Mean RT Z</strong></td>
<td>-0.0 (-0.0, 0.0)</td>
<td>-0.0 (-0.1, 0.0)</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>Iowa Gambling Task</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total T</strong></td>
<td>46.0 (41.0, 52.0)</td>
<td>49.0 (44.0, 56.5)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>Net 1</strong></td>
<td>48.0 (42.0, 55.0)</td>
<td>46.0 (38.0, 50.5)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Net 2</strong></td>
<td>49.0 (44.0, 52.0)</td>
<td>51.0 (46.5, 54.0)</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Net 3</strong></td>
<td>47.0 (41.0, 55.0)</td>
<td>49.0 (43.0, 55.0)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Net 4</strong></td>
<td>47.0 (40.0, 52.0)</td>
<td>52.0 (44.0, 62.0)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Net 5</strong></td>
<td>44.0 (34.0, 53.0)</td>
<td>52.0 (44.0, 58.5)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BNT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Spontaneous Z</strong></td>
<td>-0.8 (-1.4, -0.1)</td>
<td>-0.1 (-0.6, 0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cued Z</strong></td>
<td>-1.1 (-2.4, -0.5)</td>
<td>-0.2 (-1.3, 0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Memory/visuospatial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RAVLT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A1 Z</strong></td>
<td>-0.1 (-0.8, 1.0)</td>
<td>0.8 (-0.2, 2.4)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>A2 Z</strong></td>
<td>0.2 (-0.5, 1.4)</td>
<td>0.8 (0.1, 1.9)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>A3 Z</strong></td>
<td>0.3 (-0.4, 1.2)</td>
<td>1.1 (0.1, 2.0)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>A4 Z</strong></td>
<td>0.5 (-0.3, 1.2)</td>
<td>0.9 (0.2, 1.5)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>A5 Z</strong></td>
<td>0.8 (-0.2, 1.3)</td>
<td>0.9 (0.1, 1.9)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Total Z</strong></td>
<td>0.5(-0.5, 1.2)</td>
<td>1.0 (0.1, 2.0)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>B1 Z</strong></td>
<td>-0.2 (-0.8, 0.9)</td>
<td>0.4 (-0.2, 0.9)</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>A6 Z</strong></td>
<td>0.4 (-0.5, 1.1)</td>
<td>0.9 (0.3, 1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>A7 Z</strong></td>
<td>0.2 (-0.7, 1.1)</td>
<td>0.8 (-0.1, 1.7)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Recognition</strong></td>
<td>48.0 (44.3, 50.0)</td>
<td>48.0 (44.3, 49.0)</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>True positives Z</strong></td>
<td>0.7 (0.2, 1.0)</td>
<td>0.7 (0.3, 1.2)</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Logical Memory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LM1 Z</strong></td>
<td>0.7 (-0.4, 1.1)</td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>LM2 Z</strong></td>
<td>0.4 (-0.4, 1.0)</td>
<td>1.0 (0.0, 1.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LM recognition percentile</strong></td>
<td>50.0 (26.0, 63.0)</td>
<td>50.5 (24.8, 63.0)</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>RCFT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Copy Z</strong></td>
<td>0.4 (-0.9, 0.6)</td>
<td>0.2 (-0.9, 0.7)</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Copy time Z</strong></td>
<td>-0.7 (-1.2, -0.2)</td>
<td>-1.2 (-1.4, -0.5)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Immediate Z</strong></td>
<td>0.1 (-1.1, 1.0)</td>
<td>0.8 (-0.6, 2.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Delayed Z</strong></td>
<td>-0.2 (-1.3, 0.9)</td>
<td>0.6 (-0.5, 1.6)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Recognition Z</strong></td>
<td>-0.2 (-1.4, 0.5)</td>
<td>0.5 (-0.7, 1.1)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Social Cognition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RMET</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Z</strong></td>
<td>0.2 (-0.7, 0.6)</td>
<td>-0.0 (-0.7, 0.5)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

All scores are reported as median and interquartile range. All scores are standardised so lower score implies worse performance/shorter time. *Data was normally distributed and independent sample student’s T test performed. Otherwise, significance testing was performed using Mann-Whitney U test.
A comparison of neuropsychological performance of familial and sporadic ALS relatives and controls is made in Table 11-3. Familial ALS relatives made more inhibition errors than controls (p=0.001) and performed worse than controls at the final stage of the Iowa gambling task (p=0.008). Furthermore, familial ALS relatives performed worse on verbal fluency tasks and cued Boston naming test than both sporadic ALS relatives and controls. This is suggestive of higher heritability for these measures.

Finally, both relative groups performed worse than controls on measures of working memory (digit span backwards), verbal recall and episodic and visuospatial memory. Both familial and sporadic ALS relatives demonstrated lower pre-morbid intellectual functioning than controls, but only familial ALS relatives performed significantly worse on WASI-II FSIQ (p=0.020), a measure of current intellectual functioning.
Table 11-3: Significant differences in neuropsychological performance across familial ALS relatives, sporadic ALS relatives and control groups

<table>
<thead>
<tr>
<th></th>
<th>Across groups comparison</th>
<th>Between pairs comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FALS Relatives (n=96)</td>
<td>SALS Relatives (n=53)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.0 (31.0, 55.3)</td>
<td>50.0 (33.5, 62.5)</td>
</tr>
<tr>
<td>TOPF FSIQ</td>
<td>103.5 (96.4, 114.9)</td>
<td>105.4 (97.3, 111.3)</td>
</tr>
<tr>
<td>WASI-II FSIQ*</td>
<td>99.0 (90.0, 107.0)</td>
<td>101.0 (89.0, 106.5)</td>
</tr>
<tr>
<td>Verbal fluency FAS total Z</td>
<td>-0.6 (-1.1, 0.0)</td>
<td>0.2 (-0.8, 0.6)</td>
</tr>
<tr>
<td>CWIT Inhibition errors Z</td>
<td>0.4 (0.0, 0.7)</td>
<td>0.5 (-0.4, 0.7)</td>
</tr>
<tr>
<td>Digit span Backwards Z</td>
<td>-0.1 (-0.6, 0.7)</td>
<td>0.1 (-0.5, 1.0)</td>
</tr>
<tr>
<td>SART mean RT Z</td>
<td>0.0 (0.0, 0.0)</td>
<td>0.0 (0.0, 0.0)</td>
</tr>
<tr>
<td>Iowa Gambling Task Total T</td>
<td>45.5 (40.0, 52.0)</td>
<td>50.0 (42.5, 53.0)</td>
</tr>
<tr>
<td>net 4</td>
<td>46.0 (40.0, 52.0)</td>
<td>51.0 (41.0, 54.0)</td>
</tr>
<tr>
<td>net 5</td>
<td>43.5 (29.3, 51.0)</td>
<td>51.0 (38.0, 60.0)</td>
</tr>
<tr>
<td>BNT Spontaneous Z</td>
<td>-0.7 (1.4, 0.0)</td>
<td>-1.1 (-2.1, -0.3)</td>
</tr>
<tr>
<td>Cued Z</td>
<td>-0.9 (-2.3, -0.2)</td>
<td>-1.3 (-3.0, -0.9)</td>
</tr>
<tr>
<td>RAVLT Total z*</td>
<td>0.4 (-0.5, 1.2)</td>
<td>0.5 (-0.3, 1.3)</td>
</tr>
<tr>
<td>A6 Z*</td>
<td>0.3 (-0.5, 1.1)</td>
<td>0.7 (-0.5, 1.2)</td>
</tr>
<tr>
<td>A7 Z</td>
<td>0.0 (-0.8, 1.0)</td>
<td>0.4 (-0.6, 1.2)</td>
</tr>
<tr>
<td>Logical Memory LM2 Z*</td>
<td>0.4 (-0.4, 0.7)</td>
<td>0.4 (-0.7, 1.0)</td>
</tr>
<tr>
<td>RCFT Copy time Z</td>
<td>-0.7 (-1.1, -0.2)</td>
<td>-0.9 (-1.2, -0.1)</td>
</tr>
<tr>
<td>Immediate Z</td>
<td>0.3 (-1.1, 1.1)</td>
<td>-0.2 (-1.1, 0.6)</td>
</tr>
<tr>
<td>Recognition Z</td>
<td>-0.2 (-1.2, 0.6)</td>
<td>-0.3 (-1.3, 0.5)</td>
</tr>
</tbody>
</table>

FALS: Familial ALS. SALS: Sporadic ALS.
11.5. Impact of C9orf72 repeat expansion on detailed neuropsychological assessment performance

11 C9orf72 positive relatives and 122 C9orf72 negative relatives (C9orf72 kindreds [25], other familial ALS kindred [47], sporadic ALS kindred [50]) completed the full neuropsychological assessment. Relative cohorts were significantly younger than controls (p<0.001 respectively), but no difference in the age profile between C9orf72 carriers and non-carriers was detected.

Significant differences across groups were detected on measures of verbal fluency, language (Boston naming test spontaneous and cued), executive function (digit span backwards; Iowa gambling task), memory (RAVLT total, immediate, delayed; logical memory delayed; RCFT immediate) and general intellectual functioning (TOPF FSIQ, WASI-II FSIQ). Primarily, these findings were driven by impaired performance among C9orf72 negative relatives from familial ALS kindreds. However, C9orf72 positive relatives were observed to perform worse than C9orf72 negative relatives and controls on the Iowa gambling task (p=0.002). Of note, only 4 C9orf72 positive relatives completed this measure. Significant neuropsychological assessment findings across groups, by C9orf72 and kindred status, are discussed separately below.

11.5.1. Verbal fluency

On phonemic verbal fluency tasks, all familial ALS relatives (C9orf72 positive relatives, C9orf72 negative relatives from C9orf72 kindreds and C9orf72 negative relatives from other familial ALS kindreds) performed worse than controls (Figure 11-2). However, after correcting for multiple comparisons, only C9orf72 negative relatives from other familial ALS kindreds performed worse than controls (p<0.001). This group also performed worse than C9orf72 negative relatives from sporadic ALS kindreds (p=0.036). The lack of difference detected for relatives from C9orf72 kindreds may reflect the smaller cohort sizes. Finally, no differences between groups were detected on measures of semantic fluency (p=0.19).

11.5.2. Memory/working memory

On memory tasks, C9orf72 negative relatives from C9orf72 kindreds performed worse than controls for delayed recall (verbal memory [RAVLT delayed, p=0.013], episodic memory [logical memory delayed p=0.017]) (Figure 11-3). This was due in part to impaired working memory performance compared to controls (verbal memory [RAVLT immediate p=0.003], digit span backwards [p=0.008]). C9orf72 negative relatives from other familial ALS kindreds also performed worse than controls on digit span backwards
(p=0.032), indicating the presence of working memory difficulties in this cohort also. Finally, C9orf72 negative relatives from sporadic ALS kindreds were found to perform worse on visual memory immediate recall (RCFT, p=0.003). No differences were detected for delayed recall or figure copying on this task.

11.5.3. Language

C9orf72 negative relatives from sporadic ALS kindreds performed worse than controls on the Boston Naming Test (spontaneous [p<0.001], cued [p<0.001]) (Figure 11-4). C9orf72 negative relatives from familial ALS kindreds (not known to carry any ALS gene mutation) also performed worse than controls on this measure (Boston Naming Test spontaneous [p=0.001], cued [p<0.001]). No other differences between groups were detected.

11.5.4. Iowa Gambling Task

C9orf72 positive relatives performed worse than all other relative groups and controls on the Iowa gambling task (p=0.004). In particular, after controlling for multiple comparisons, they were found to perform significantly worse than C9orf72 negative relatives from other familial ALS kindreds (p=0.025) and controls (p=0.006) (Figure 11-5). However, the numbers of C9orf72 positive who completed this task was very low (n=4). No significant differences were detected between C9orf72 negative relatives from C9orf72 kindreds and other groups.

11.5.5. Intellectual Functioning

C9orf72 negative relatives from C9orf72 kindreds performed worse than controls on measures of intellectual functioning (TOPF FSIQ [p=0.004], WASI-II FSIQ [p=0.037]) (Figure 11-6). On the WASI-II task, these differences were attributable to impaired performance on vocabulary paradigm (p<0.001), rather than matrix reasoning task (p=0.065). No other significant differences on intellectual functioning were detected between groups.
♦ C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♠ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.

<table>
<thead>
<tr>
<th></th>
<th>C9orf72 positive relatives (FALS*) (n=11)</th>
<th>C9orf72 negative relatives (FALS*) (n=25)</th>
<th>C9orf72 negative relatives (FALS*) (n=47)</th>
<th>C9orf72 negative relatives (SALS) (n=50)</th>
<th>Controls (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS total Z</td>
<td>-0.4 (-1.2, 0.2)</td>
<td>-0.6 (-1.1, 0.4)</td>
<td>-0.6 (-1.1, -0.2)</td>
<td>0.1 (-0.8, 0.6)</td>
<td>0.1 (-0.5, 1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Animals total Z</td>
<td>0.4 (-0.5, 0.9)</td>
<td>-0.6 (-0.9, -0.1)</td>
<td>-0.3 (-1.1, 0.5)</td>
<td>-0.1 (-0.5, 0.8)</td>
<td>-0.2 (-0.8, 0.7)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Figure 11-2: Comparison of verbal fluency performance across C9orf72 positive relatives, C9orf72 negative relatives and controls
C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ● Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.

<table>
<thead>
<tr>
<th></th>
<th>C9orf72 positive relatives (FALS*)</th>
<th>C9orf72 negative relatives (FALS*)</th>
<th>C9orf72 negative relatives (SALS)</th>
<th>Controls (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span Backwards Z</td>
<td>0.1 (-0.6, 1.2)</td>
<td>-0.1 (-0.9, 0.5)</td>
<td>0.1 (-0.6, 1.0)</td>
<td>0.5 (0.0, 1.4)</td>
<td>0.004</td>
</tr>
<tr>
<td>RAVLT Total z</td>
<td>0.3 (-0.0, 1.2)</td>
<td>0.2 (-0.9, 1.3)</td>
<td>0.7 (-0.5, 1.2)</td>
<td>1.0 (0.1, 2.0)</td>
<td>0.034</td>
</tr>
<tr>
<td>RAVLT Immediate Z</td>
<td>0.8 (-0.2, 1.1)</td>
<td>0.2 (-0.9, 0.6)</td>
<td>0.4 (-0.5, 1.3)</td>
<td>0.9 (0.3, 1.8)</td>
<td>0.008</td>
</tr>
<tr>
<td>RAVLT Delayed Z</td>
<td>0.6 (-0.4, 1.2)</td>
<td>-0.5 (-1.1, 0.6)</td>
<td>0.0 (-0.9, 1.2)</td>
<td>0.8 (-0.1, 1.7)</td>
<td>0.012</td>
</tr>
<tr>
<td>LM Delayed Z</td>
<td>0.4 (-0.4, 1.0)</td>
<td>0.0 (-1.0, 0.7)</td>
<td>0.4 (0.0, 0.7)</td>
<td>1.0 (0.0, 1.4)</td>
<td>0.009</td>
</tr>
<tr>
<td>RCFT Immediate Z</td>
<td>0.5 (-1.2, 1.9)</td>
<td>0.2 (-0.8, 0.9)</td>
<td>0.6 (-1.1, 1.3)</td>
<td>0.8 (-0.6, 2.0)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Figure 11-3: Comparison of memory task performance across C9orf72 positive relatives, C9orf72 negative relatives and controls.
C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♦ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.

<table>
<thead>
<tr>
<th>C9orf72 positive relatives (FALS*)</th>
<th>C9orf72 negative relatives (FALS*)</th>
<th>C9orf72 negative relatives (SALS)</th>
<th>Controls (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous Z</td>
<td>-0.3 (-1.3, 0.2)</td>
<td>-0.3 (-1.1, 0.0)</td>
<td>-1.0 (-2.0, -0.3)</td>
<td>-0.1 (-0.6, 0.3)</td>
</tr>
<tr>
<td>Cued Z</td>
<td>-0.1 (-1.3, -0.1)</td>
<td>-0.7 (-1.3, -0.1)</td>
<td>-1.3 (-3.0, -0.8)</td>
<td>-0.2 (-1.3, 0.3)</td>
</tr>
</tbody>
</table>

Figure 11-4: Comparison of Boston Naming Test performance across C9orf72 positive relatives, C9orf72 negative relatives and controls
C9orf72 positive relatives (FALS*)
(n=4)

C9orf72 negative relatives (FALS*)
(n=6)

C9orf72 negative relatives (FALS*)
(n=29)

C9orf72 negative relatives (SALS)
(n=13)

Controls
(n=41)

<table>
<thead>
<tr>
<th></th>
<th>Total T</th>
<th>Net 1</th>
<th>Net 2</th>
<th>Net 3</th>
<th>Net 4</th>
<th>Net 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30.5 (24.0, 34.0)</td>
<td>43.5 (35.0, 46.8)</td>
<td>49.0 (44.0, 54.5)</td>
<td>50.0 (42.5, 53.0)</td>
<td>49.0 (44.0, 56.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Net 1</td>
<td>35.5 (29.5, 43.0)</td>
<td>45.0 (43.8, 55.5)</td>
<td>48.0 (37.0, 58.0)</td>
<td>48.0 (46.0, 52.5)</td>
<td>46.0 (38.0, 50.5)</td>
<td>0.14</td>
</tr>
<tr>
<td>Net 2</td>
<td>43.0 (39.8, 45.5)</td>
<td>44.5 (42.5, 54.5)</td>
<td>52.0 (46.5, 55.0)</td>
<td>46.0 (44.0, 49.0)</td>
<td>51.0 (46.5, 54.0)</td>
<td>0.016</td>
</tr>
<tr>
<td>Net 3</td>
<td>29.5 (22.0, 43.8)</td>
<td>43.5 (39.3, 49.0)</td>
<td>51.0 (43.5, 55.5)</td>
<td>50.0 (39.0, 56.0)</td>
<td>49.0 (43.0, 55.0)</td>
<td>0.076</td>
</tr>
<tr>
<td>Net 4</td>
<td>30.5 (20.0, 42.5)</td>
<td>43.5 (41.5, 48.8)</td>
<td>48.0 (42.5, 56.5)</td>
<td>51.0 (41.0, 54.0)</td>
<td>52.0 (44.0, 62.0)</td>
<td>0.047</td>
</tr>
<tr>
<td>Net 5</td>
<td>20.0 (19.0, 43.5)</td>
<td>31.5 (20.5, 49.3)</td>
<td>45.0 (37.0, 56.0)</td>
<td>51.0 (38.0, 60.0)</td>
<td>52.0 (44.0, 58.5)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Figure 11-5: Comparison of Iowa Gambling Task performance across C9orf72 positive relatives, C9orf72 negative relatives and control.

♦ C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♦ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.
C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♦ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband

<table>
<thead>
<tr>
<th></th>
<th>C9orf72 positive relatives (FALS*) (n=11)</th>
<th>C9orf72 negative relatives (FALS*) (n=25)</th>
<th>C9orf72 negative relatives (FALS*) (n=46)</th>
<th>C9orf72 negative relatives (SALS) (n=50)</th>
<th>Controls (n=58)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOPF FSIQ</td>
<td>109.4 (99.1, 119.4)</td>
<td>98.0 (92.5, 107.7)</td>
<td>105.0 (96.7, 118.2)</td>
<td>105.4 (96.6, 110.8)</td>
<td>109.3 (100.1, 121.2)</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>WASI-II FSIQ</td>
<td>105.0 (99.0, 109.0)</td>
<td>95.0 (87.0, 105.0)</td>
<td>101.0 (87.0, 107.0)</td>
<td>101.0 (89.0, 106.0)</td>
<td>105.5 (89.8, 115.0)</td>
<td><strong>0.026</strong></td>
</tr>
</tbody>
</table>

Figure 11-6: Comparison of Intellectual Functioning performance across C9orf72 positive relatives, C9orf72 negative relatives and controls
11.6. **Summary of findings**

A summary of the key findings from this study exploring the performance of ALS relatives and healthy controls on a comprehensive neuropsychological assessment are outlined below. All neuropsychological data is age-standardised allowing for comparison across groups. All relative and control groups were matched for years of education.

1. Relatives performed worse than controls across multiple cognitive domains, including verbal fluency, language, executive function and memory.

2. These findings were driven by familial ALS relatives who performed worse than controls on verbal fluency, language, executive function and general intelligence. Familial relatives also performed worse than sporadic relatives on verbal fluency and cued Boston naming.

3. The assessment of C9orf72 positive relatives was limited by small numbers, with carriers less likely to complete the comprehensive neuropsychological assessment. The vast majority (8/11) of C9orf72 were under 45 years of age. C9orf72 positive relatives performed worse than both C9orf72 negative relatives and controls on the Iowa gambling task, although this may be an artifact of small numbers (n=4).

4. C9orf72 negative relatives from both C9orf72 kindreds and other familial ALS kindreds performed poorly on measures of verbal fluency and working memory. Both groups also showed some impairment on language predominant tasks (C9orf72 kindreds [WASI vocabulary]; other FALS [Boston naming test]).

11.7. **Discussion**

This detailed and comprehensive neuropsychology study has verified and expanded on the findings from the ECAS study, using age-standardised measures. Both studies align in demonstrating the presence of widespread but subtle cognitive impairment among relatives of people with ALS. In particular, both studies demonstrated familial clustering of verbal fluency deficits, independent of C9orf72 status and exceeding that observed among sporadic ALS relatives. This pattern designates this measure as the most promising putative cognitive endophenotype in ALS.

Verbal fluency difficulties are the most consistent cognitive deficit observed in patients with ALS and, as with this study, these deficits are consistently more evident on phonemic rather than semantic paradigms. In ALS patients with executive dysfunction,
abnormal phonemic verbal fluency is nearly ubiquitous, with 30% of patients with cognitive impairment without executive dysfunction also displaying impaired performance on this measure. In ALS patient cohorts, these phonemic verbal fluency deficits have been shown to correspond with involvement of the frontal-striatal connections, particularly the dorsolateral prefrontal cortex and anterior cingulate areas. This would suggest these deficits relate more to executive rather than linguistic processes, which aligns with work by Abrahams et al (2000) who showed that verbal fluency deficits in ALS primarily result from higher order executive dysfunction rather than impaired phonological loop circuits or a simple word retrieval deficits.

Phonemic verbal fluency scores have been shown to remain stable in some longitudinal studies of neuropsychological function in ALS, compared to measures of semantic verbal fluency which were shown to decline. These findings mirror work by Mathuranath et al (2003) who showed phonemic verbal fluency performance was age-independent, in contrast to semantic verbal fluency which was not. Nonetheless, the apparent temporal stability of phonemic fluency in ALS could also result from bias introduced to longitudinal studies in ALS by attrition patterns where patients with greater physical and cognitive well-being are more likely to complete all assessment timepoints. Temporal stability is an important criterion for putative endophenotypes, as infers that the measure is a state independent marker of genetic risk, rather than a biomarker of disease progression. Notably, the C9orf72 positive relatives demonstrated decline on this measure across increasing age-categories on the ECAS study (Chapter 10). Given the presence of similar deficits in C9orf72 negative relatives from the same and other familial ALS kindreds, this decline could reflect an active disease process interacting with other oligogenic risk.

Familial ALS relatives were also found to perform worse than controls on measures of executive function and language. Similar findings have been inconsistently reported for asymptomatic C9orf72 carriers. Rohrer et al (2015) detected changes on verbal fluency, executive function and language tasks up to 15 years before expected disease onset in mutation carriers. However, these findings were not replicated in several subsequent studies, comparing neuropsychological functioning in expansion carriers and familial non-carriers. The discrepancies in these findings may be explained in part by the novel observation in this project that C9orf72 negative relatives from both C9orf72 kindreds and other familial ALS kindreds also demonstrated evidence of executive and language impairment compared with healthy controls. These findings, together with the observations that C9orf72 positive FTD ± ALS patients perform worse
than asymptomatic gene carriers on similar tasks, suggests the existence of a phenotypic spectrum in cognitive changes corresponding to degree of genetic liability.

Interestingly, C9orf72 negative relatives from C9orf72 kindreds were also found to perform worse than controls on some measures of immediate (episodic) and delayed (verbal and episodic) memory. However, in light of the other impairments detected in this cohort, it is difficult to determine the exact nature of these deficits. This issue has also arisen previously in ALS patient cohorts. At present, isolated memory impairment is not deemed sufficient to meet diagnostic criteria for ALSci (Strong, 2017), with the authors of these criteria acknowledging that memory deficits in ALS rarely occur in isolation. In general, Alzheimer’s dementia is much less common among ALS patients compared to the frontotemporal dementias, with an estimated prevalence of the former approximating 1% in ALS cohorts compared to 13% for the latter. While both disorders have distinct pathological features, they share some common features of neurodegeneration including neuroinflammation, autophagy, and oxidative stress. Nonetheless, the pattern of memory deficits in ALS patients (when present) appears qualitatively distinct from patients with Alzheimer’s disease. Further work comprehensively exploring the processes of encoding, storage, recall, processing speed, and recognition processes, is required to delineate the nature of memory deficits detected among C9orf72 negative relatives. Yet, it seems probable that these deficits will most likely be attributable to declines in some combination of attention, language, and executive functioning domains.

Furthermore, in this study, the C9orf72 positive relatives performed worst on the Iowa Gambling Task, a measure of decision making, working memory, risk discernment, and emotional processing. This mirrors findings in both ALS and bvFTD cohorts where patients demonstrated impaired capacity in learning to avoid disadvantageous stimuli. While this task was primarily designed to detect dysfunction arising in the ventromedial prefrontal cortex, multiple cortical and subcortical regions (including the orbitofrontal and dorsolateral prefrontal cortex, frontal pole, cingulate, parietal and temporal cortex, cerebellum, putamen, amygdala and hippocampus) have since been linked to impaired performance on this measure. While the findings of this study have potential to novel provide insights into the extent of neural network dysfunction among asymptomatic C9orf72 carriers, in light of the low number of recruits, they must be interpreted with caution. In particular, given the reported high inter-individual variability on this task, it is feasible that this signal may not persist in larger cohort.
11.7.1. **Limitations**

Some limitations of this study have already been discussed in Chapter 10 as participants in this study predominantly correspond with those assessed in the ECAS study. Moreover, subtle difference in the cohorts could potentially bias to this study’s findings. Participants who scored lower on ECAS ALS non-specific tasks were less likely to complete this more detailed assessment, implying that deficits detected on memory tasks on this study may be conservatively estimated. Equally, over 30% of non-participants were C9orf72 carriers, further limiting our ability to comprehensively assess this cohort. Finally, it is notable that C9orf72 negative relatives from C9orf72 kindreds performed worse than controls on measures of general intellectual functioning. While this would not account for the deficits observed in other relative cohorts, it could partially explain the deficits detected in this C9orf72 negative relative cohort.

Yet, it is worth noting that general intelligence could also be considered a putative cognitive endophenotype for ALS. The heritability of this measures is reported as moderate to high\(^{545}\) and there is increasing evidence that ALS risk is negative correlated with higher general cognitive performance, verbal and numeric reasoning and educational attainment\(^{358, 531}\). Furthermore, intelligence testing is replete with hidden assumptions and biases. This study’s homogenous study population is unlikely to have introduced significant cultural bias. Yet, in light of the genetic overlap between ALS and other neuropsychiatric disorders, the knowledge that intelligence tests also favour neurotypical individuals may raise more concerns. Further work is required to explore the relationship between intellectual functioning and ALS risk.

11.7.2. **Conclusion**

The findings of this chapter expand on those of the ECAS study, verifying the presence of extensive but subtle cognitive changes among relatives of those with ALS, and demonstrating convergent and divergent phenotypic patterns compared with those reported for asymptomatic C9orf72 repeat expansion carriers. In particular, phonemic verbal fluency was found to cluster in familial ALS kindreds not accounted for by the pathogenic repeat expansion indicating that early vulnerabilities of frontostriatal networks may be induced by ALS genetic risk. Similar deficits are suggested as putative endophenotypes for ALS associated neuropsychiatric disorders\(^{367}\) further supporting the hypothesis that these disorders exist on a phenotypic spectrum with ALS, reflecting their shared genetic risk.
12. Chapter 12: Results Part VIII: Comparison of Neuropsychiatric Traits of Relatives of People with ALS and Healthy Controls

12.1. Introduction

As discussed in previous chapters, several studies have reported on a higher incidence of neuropsychiatric disorders among relatives of people with ALS\textsuperscript{314,354,355}. These studies have been limited to proxy reports of disorders, usually by the proband. This study explores this phenomenon further by directly assessing neuropsychiatric traits of asymptomatic relatives of people with ALS and healthy controls (Project Aim 3). Study methodology procedures, including details of the full neuropsychiatric battery used, are outlined in Chapters 4 and 10 respectively. The results of this study are reported below.

Results

12.2. Demographics

All participants who completed ECAS +/- full neuropsychological assessment were offered the opportunity to complete an online neuropsychiatric trait assessment. 147 relatives (familial [104], sporadic [43]) and 69 controls completed the assessment. In the familial ALS relative cohort, 25 relatives carrying the pathogenic C9orf72 repeat expansion completed the assessment along with 48 non-carriers from the same kindreds. An additional 22 C9orf72 negative relatives from other familial ALS kindreds (not known to carry any pathogenic ALS mutation) took part in the study. Finally, 40 C9orf72 negative relatives from sporadic ALS kindreds completed the assessment. Relative and control groups were matched for sex (p=0.83) and education level (p=0.84), but relatives were younger at time of assessment than controls (p<0.001).

12.2.1. Participants and non-participants

Relatives who did not complete the online neuropsychiatric traits assessment performed worse than those who did on ECAS ALS-specific tasks (p=0.028), particularly verbal fluency (p=0.016) (Table 12-1). Otherwise, there were no differences between the groups for sex, age, education, familial status and C9orf72 status.
Table 12-1: Comparison of participants and non-participants for online neuropsychiatric trait assessment

<table>
<thead>
<tr>
<th></th>
<th>Completed assessment (n=147)</th>
<th>Did not complete assessment (n=66)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics (no., %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>60.0 (40.8)</td>
<td>36.0 (54.5)</td>
<td>0.070</td>
</tr>
<tr>
<td>C9orf72 (positive)</td>
<td>25.0 (17.4)</td>
<td>6.0 (10.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>FALS relative (yes)</td>
<td>104.0 (70.7)</td>
<td>51.0 (77.3)</td>
<td>0.39</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.5 (33.0, 57.0)</td>
<td>40.0 (27.8, 59.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>Education (years)</td>
<td>16.0 (14.0, 19.0)</td>
<td>17.0 (14.5, 19.0)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Performance on ECAS cognitive screen (median, IQR)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECAS total</td>
<td>112.0 (104.0, 118.3)</td>
<td>110.0 (94.5, 117.0)</td>
<td>0.083</td>
</tr>
<tr>
<td>ECAS ALS specific</td>
<td>85.0 (77.0, 88.0)</td>
<td>81.0 (70.0, 86.5)</td>
<td>0.028</td>
</tr>
<tr>
<td>ECAS ALS non-specific</td>
<td>29.0 (26.3, 31.0)</td>
<td>29.0 (26.0, 31.0)</td>
<td>0.83</td>
</tr>
<tr>
<td>Language</td>
<td>27.0 (26.0, 28.0)</td>
<td>26.0 (25.0, 28.0)</td>
<td>0.12</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>20.0 (18.0, 20.0)</td>
<td>18.0 (13.0, 20.0)</td>
<td>0.016</td>
</tr>
<tr>
<td>Executive</td>
<td>39.0 (35.0, 42.0)</td>
<td>37.5 (32.8, 41.0)</td>
<td>0.095</td>
</tr>
<tr>
<td>Memory</td>
<td>18.0 (15.0, 19.0)</td>
<td>18.0 (14.8, 20.0)</td>
<td>0.86</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>12.0 (11.3, 12.0)</td>
<td>12.0 (11.0, 12.0)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Lower score on ECAS indicate worse performance.

12.3. Comparison of neuropsychiatric traits of relatives and controls

Relatives scored worse than controls on measures of depression, anxiety, psychosis risk, apathy and autistic traits (Table 12-2). Applying pathological cut-offs\(^{481}\), 31/141 (22.2%) of relatives were at high psychotic risk compared with 5/69 (7.2%) of controls (p=0.007). Similarly, a greater proportion of relatives exhibited significant apathy\(^{472}\) compared with controls (63.8% v 47.8%, [p=0.027]). No differences between groups with respect to the proportions of participants with pathological depression or anxiety were detected. Finally, relatives exhibited greater tendencies towards “disorganized, careless” traits and “conventional, uncreative” traits on the Ten-Item Personality Inventory (TIPI).
Table 12-2: Comparison of Neuropsychiatric Traits Assessment performance among relatives of ALS patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Relative (n=147)</th>
<th>Controls (n=69)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHQ total</td>
<td>2.0 (0.0, 5.0)</td>
<td>1.0 (0.0, 3.0)</td>
<td>0.009</td>
</tr>
<tr>
<td>GAD total</td>
<td>2.0 (0.0, 4.0)</td>
<td>0.0 (0.0, 2.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>CAPE total*</td>
<td>1.1 (1.0, 1.1)</td>
<td>1.0 (1.0, 1.1)</td>
<td>0.016</td>
</tr>
<tr>
<td>OCIR total</td>
<td>4.0 (1.0, 9.0)</td>
<td>5.0 (2.0, 9.0)</td>
<td>0.55</td>
</tr>
<tr>
<td>AQ Total</td>
<td>24.0 (20.0, 27.0)</td>
<td>22.0 (19.0, 26.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>AQ social skill</td>
<td>3.0 (3.0, 4.0)</td>
<td>3.0 (3.0, 4.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>AQ attention switching</td>
<td>4.0 (3.0, 5.0)</td>
<td>4.0 (3.0, 5.0)</td>
<td>0.91</td>
</tr>
<tr>
<td>AQ attention to detail</td>
<td>6.0 (5.0, 7.0)</td>
<td>5.0 (4.0, 6.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>AQ communication</td>
<td>6.0 (5.0, 6.0)</td>
<td>6.0 (5.0, 6.0)</td>
<td>0.58</td>
</tr>
<tr>
<td>AQ imagination</td>
<td>4.0 (3.0, 5.0)</td>
<td>4.0 (3.5, 6.0)</td>
<td>0.32</td>
</tr>
<tr>
<td>DAS Total</td>
<td>41.0 (36.5, 45.0)</td>
<td>38.0 (35.0, 43.0)</td>
<td>0.027</td>
</tr>
<tr>
<td>DAS executive</td>
<td>16.0 (12.0, 18.5)</td>
<td>15.0 (13.0, 18.0)</td>
<td>0.76</td>
</tr>
<tr>
<td>DAS emotional*</td>
<td>13.0 (11.0, 15.0)</td>
<td>13.0 (11.0, 15.0)</td>
<td>0.78</td>
</tr>
<tr>
<td>DAS initiation*</td>
<td>11.0 (9.0, 14.0)</td>
<td>9.0 (7.5, 12.0)</td>
<td>0.013</td>
</tr>
<tr>
<td>ARSR Total</td>
<td>1.0 (0.0, 2.0)</td>
<td>1.0 (0.0, 3.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>BIS Total*</td>
<td>55.0 (49.5, 60.0)</td>
<td>54.0 (49.0, 61.0)</td>
<td>0.40</td>
</tr>
<tr>
<td>BIS attention</td>
<td>12.0 (10.0, 14.0)</td>
<td>12.0 (10.0, 14.0)</td>
<td>0.87</td>
</tr>
<tr>
<td>BIS motor*</td>
<td>21.0 (18.0, 23.0)</td>
<td>20.0 (18.0, 23.0)</td>
<td>0.68</td>
</tr>
<tr>
<td>BIS non-planning</td>
<td>23.0 (20.0, 26.0)</td>
<td>22.0 (19.0, 25.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>TIPI extraversion</td>
<td>4.0 (4.0, 5.0)</td>
<td>4.5 (4.0, 5.5)</td>
<td>0.10</td>
</tr>
<tr>
<td>TIPI agreeableness</td>
<td>4.5 (4.0, 5.5)</td>
<td>4.5 (4.0, 5.0)</td>
<td>0.22</td>
</tr>
<tr>
<td>TIPI conscientiousness</td>
<td>4.0 (3.5, 4.5)</td>
<td>4.0 (4.0, 5.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>TIPI emotional stability</td>
<td>4.5 (4.0, 5.5)</td>
<td>4.5 (4.0, 5.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>TIPI openness to experience</td>
<td>4.0 (3.5, 5.0)</td>
<td>4.5 (4.0, 5.5)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* normally distributed. a. Total score is weighed by number of questions answered. The median and interquartile range of raw total scores for each test are reported above. For each test, a higher score implies more severe symptoms. TIPI tests are scored from 1-7, with higher scores reflecting greater similarity with the characteristic described.

12.4. Comparison of neuropsychiatric traits of familial ALS relatives, sporadic ALS relatives and controls

Comparing relatives from familial and sporadic ALS kindreds and controls, differences were evident across groups for multiple neuropsychiatric measures (Table 12-3). For the most part, the distinctions were driven by differences between familial ALS relatives and controls (anxiety [p=0.002], psychosis risk [p=0.008], initiation apathy [p=0.041], ‘openness to experience’ traits [p=0.003]).

However, both familial and sporadic ALS relatives were more ‘detail orientated’ and ‘disorganised’ compared with controls (AQ attention to detail [p=0.004], TIPI conscientiousness [p=0.010]). Only psychosis risk was found to vary significantly between the groups when pathological cut-offs were applied, with 24/99 (24.2%) of
familial ALS relatives, 7/42 (16.7%) of sporadic ALS relatives and 5/69 (7.2%) controls at increased risk of psychosis (p=0.016).

12.4.1. Impact of C9orf72 repeat expansion

Neuropsychiatric trait assessments were completed by 25 C9orf72 positive relatives and 110 C9orf72 negative relatives (C9orf72 kindreds [48], other familial ALS kindred [22], sporadic ALS kindred [40]). Relative cohorts were significantly younger than controls (p<0.001 respectively), but no difference in the age profile between C9orf72 carriers and non-carriers was detected.

The differences above were driven by C9orf72 negative relatives from C9orf72 kindreds who performed worse than controls across multiple measures of neuropsychiatric function including depression (p=0.024), anxiety (p=0.005), psychosis risk (p=0.001), initiation apathy (p=0.030) and showed less ‘openness to experience’ traits (p=0.002). No differences were found between C9orf72 positive relatives and other groups, although this group was limited by small numbers.

A greater proportion of C9orf72 positive relatives, C9orf72 negative relatives from the same kindreds and C9orf72 negative relatives from sporadic ALS relatives were at high psychosis risk (25.0%, 31.1% and 17.9% respectively) compared to C9orf72 negative relatives from other familial ALS kindreds and controls (5.0% and 7.2% respectively) (p=0.006). No other differences were found between groups when pathological cut-offs were applied.
Table 12-3: Significant differences in neuropsychiatric traits across familial ALS relatives, sporadic ALS relatives and controls

<table>
<thead>
<tr>
<th>Trait</th>
<th>Across groups comparison</th>
<th>Between pairs comparison</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FALS Relatives (n=104)</td>
<td>SALS Relatives (n=43)</td>
<td>Controls (n=69)</td>
</tr>
<tr>
<td>PHQ total</td>
<td>2.0 (0.0, 5.0)</td>
<td>2.0 (1.0, 5.3)</td>
<td>1.0 (0.0, 3.0)</td>
</tr>
<tr>
<td>GAD-7 total</td>
<td>2.0 (0.0, 4.0)</td>
<td>0.5 (0.0, 4.0)</td>
<td>0.0 (0.0, 2.8)</td>
</tr>
<tr>
<td>CAPE-P15 total*</td>
<td>1.1 (1.1, 1.1)</td>
<td>1.1 (1.0, 1.1)</td>
<td>1.0 (1.0, 1.1)</td>
</tr>
<tr>
<td>AQ total</td>
<td>24.0 (20.0, 26.8)</td>
<td>24.0 (20.0, 27.0)</td>
<td>22.0 (19.0, 26.0)</td>
</tr>
<tr>
<td>AQ attention to detail</td>
<td>6.0 (5.0, 7.0)</td>
<td>6.0 (5.0, 7.0)</td>
<td>5.0 (4.0, 6.0)</td>
</tr>
<tr>
<td>DAS total</td>
<td>41.0 (36.0, 45.0)</td>
<td>40.0 (37.0, 44.0)</td>
<td>38.0 (35.0, 43.0)</td>
</tr>
<tr>
<td>DAS Initiation*</td>
<td>11.0 (9.0, 14.0)</td>
<td>11.0 (9.0, 13.5)</td>
<td>9.0 (7.5, 12.0)</td>
</tr>
<tr>
<td>TIPI conscientiousness</td>
<td>4.0 (3.5, 4.5)</td>
<td>4.0 (3.8, 4.3)</td>
<td>4.0 (4.0, 5.5)</td>
</tr>
<tr>
<td>TIPI openness to experience</td>
<td>4.0 (3.5, 5.0)</td>
<td>4.5 (3.5, 5.0)</td>
<td>4.5 (4.0, 5.5)</td>
</tr>
</tbody>
</table>
12.5. **Controlling for impact of age on neuropsychiatric traits**

To control for the impact of age on the neuropsychiatric traits across familial relatives, sporadic relatives and control groups, participants were sub-categorised into four groups based on their age at the time of assessment (<45, 45-54, 55-64 and 65+ years). Included in the under 45 years age category were 65 familial ALS relatives, 15 sporadic ALS relatives and 4 controls. Among participants aged 45-54 years were 14 familial ALS relatives, 7 sporadic ALS relatives and 10 controls. There were 15 familial ALS relatives, 10 sporadic ALS relatives and 22 controls in the 55–64-year-old age category. Finally, 10 familial ALS relatives, 10 sporadic ALS relatives and 33 controls were aged 65 years or older at the time of the assessment. The vast majority of relatives from C9orf72 positive kindreds were aged less than 45 years, with the majority of controls aged 65 years or more (Table 12-4).

<table>
<thead>
<tr>
<th>Age Category (years)</th>
<th>&lt;45</th>
<th>45-54</th>
<th>55-64</th>
<th>65+</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9orf72 positive relative (FALS♦)</td>
<td>17</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>C9orf72 negative relative (FALS♦)</td>
<td>32</td>
<td>4</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>C9orf72 negative relative (FALS♠)</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>C9orf72 negative relative (SALS)</td>
<td>13</td>
<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>10</td>
<td>22</td>
<td>34</td>
</tr>
</tbody>
</table>

♦ C9orf72 Familial ALS kindreds: at least one ALS proband carries the C9orf72 repeat expansion. ♠ Familial ALS kindreds (Genetically-undetermined): no pathogenic mutation identified in any ALS proband.

Most between group differences on neuropsychiatric traits did not withstand correction for age, although this may represent a loss of power with the sub-categorisation. Significantly higher levels of anxiety and psychosis risk were detected only among familial ALS relatives aged 65 years or older. No significant difference between groups for any age category were detected for depression, ADHD, apathy, impulsiveness, social skills, attention to detail, imagination, extraversion, agreeableness, conscientiousness and neuroticism. The impact of age on neuropsychiatric traits is further explored in relation to C9orf72 positive and negative relatives from the same kindred below.

12.6. **C9orf72 positive relatives**

Assessment of this cohort was limited by small numbers, especially in the older age categories. However, among those aged under 45 years, C9orf72 positive relatives
showed greater levels of self-control (BIS [p=0.017]), “cognitive complexity” (BIS [p=0.034]) and extraversion (TIPI [p=0.023]) compared with C9orf72 negative relatives. Cognitive complexity refers to a non-planning sub-score of the Barrett Impulsiveness Scale (BIS). This difference reflects that C9orf72 positive relatives were more likely to answer in a positive manner to questions including “I like to think about complex problem”, “I like puzzles” and “I save regularly” and more likely to answer in a negative manner to questions including “I am more interested in the present than in the future” and “I get easily bored when solving thought problems”.

Interestingly, levels of self-control were found to decline across increasing age categories (p=0.004), such that in the 54–65-year-old age category C9orf72 positive relatives demonstrated the lowest levels of self-control (Figure 12-1). No differences across age categories were observed for BIS cognitive complexity (p=0.71) or TIPI extraversion (p=0.22). Finally, no differences were observed between C9orf72 positive versus negative relatives for any age category on measures of depression, anxiety, psychosis risk, obsessionality, apathy, attention deficit and other personality traits.
BIS self-control: high scores imply worse self-control. BIS Cognitive complexity: high scores imply less cognitive complexity. TIPI Extraversion: high scores imply more extraverted. Controls were excluded from the analysis due to the low numbers aged less than 45 years. Only one C9orf72 positive over 65 years completed the analysis and so they were excluded.

<table>
<thead>
<tr>
<th>C9orf72 relative</th>
<th>BIS Self-control</th>
<th>BIS complexity</th>
<th>Cognitive</th>
<th>TIPI Extraversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;45 years</td>
<td>10.0 (8.0, 13.0)</td>
<td>9.5 (9.0, 12.8)</td>
<td>4.0 (4.0, 5.0)</td>
<td></td>
</tr>
<tr>
<td>45-54 years</td>
<td>13.0 (12.3, 14.5)</td>
<td>11.0 (8.5, 13.5)</td>
<td>4.3 (3.3, 5.3)</td>
<td></td>
</tr>
<tr>
<td>55-64 years</td>
<td>16.0 (11.0, 16.0)</td>
<td>9.0 (9.0, 9.0)</td>
<td>4.0 (3.5, 4.0)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 12-1: C9orf72 positive relative performance on Barrett Impulsiveness (BIS) Self-Control and Cognitive Complexity and Ten Item Personality Inventory Extraversion
12.7. Corf72 negative relatives from C9orf72 kindreds

48 C9orf72 negative relatives from C9orf72 kindreds, completed the neuropsychiatric traits assessment, with the vast majority aged less than 45 years (32/48 [66.6%]). Only those aged 65 years or older, demonstrated higher levels of anxiety (p=0.033) and less openness to experience (p=0.034) compared with controls (Figure 12-2). A trend towards less openness to experience was also noted in C9orf72 negative relatives from C9orf72 kindreds aged between 55 to 64 years (p=0.057) compared with controls. However, for both measures, no worsening of symptoms across increasing age groups was detected (GAD-7 p=0.35; TIPI openness p=0.12).

Higher scores represent more severe symptoms (GAD-7) and greater openness to experience (TIPI).
### 12.8. Summary of findings

A summary of the key findings from this study exploring the neuropsychiatric trait profiles of ALS relatives and healthy controls are outlined below.

1. Relatives reported higher levels of neuropsychiatric dysfunction than controls across many measures including depression, anxiety, psychosis risk, apathy and autistic traits, with a higher proportion of relatives at high risk of developing psychosis. Relatives were more disorganized, and less open to experience on tests of personality.

2. Overall, familial relatives demonstrated higher levels of anxiety, psychosis risk, initiation apathy and were less open to experience compared with controls, with a high proportion of familial relatives at high risk of psychosis. These differences were driven by C9orf72 negative relatives from C9orf72 kindreds. However, after controlling for differences in age profiles across cohorts, these distinctions were less clear.

3. C9orf72 positive relatives who completed this assessment were young, with the vast majority being under 45 years of age. In this age category, C9orf72 positive relatives were found to report higher levels of extraversion, self-control and “cognitive complexity” compared with C9orf72 negative relatives. Levels of self-control were found to decline across increasing age categories, such that C9orf72 positive relatives aged between 55-64 years showed the lowest levels of self-control of any cohort.

4. After controlling for age, C9orf72 negative relatives from C9orf72 kindreds only showed higher levels of anxiety and reduced openness to experience for those aged 65 years or older. A trend towards reduced openness to experience was also noted in those age between 55-64 years.

---

<table>
<thead>
<tr>
<th>Age category (years)</th>
<th>&lt;45</th>
<th>45-54</th>
<th>55-64</th>
<th>65+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety (GAD-7)</td>
<td>3.0 (0.0, 6.0)</td>
<td>1.0 (0.0, 2.8)</td>
<td>2.0 (0.0, 4.0)</td>
<td>5.0 (1.5, 11.5)</td>
</tr>
<tr>
<td>Openness to experience (TIPI)</td>
<td>4.0 (3.5, 5.0)</td>
<td>4.3 (2.5, 6.0)</td>
<td>3.5 (3.0, 4.0)</td>
<td>3.5 (1.8, 4.0)</td>
</tr>
</tbody>
</table>

*Figure 12-2: Performance of C9orf72 negative relatives from C9orf72 kindreds, by age category, on Generalised Anxiety Disorder-7 (GAD-7) and Ten Item Personality Inventory (TIPI) Openness to Experience*
12.9. Discussion

This is the largest study performed to date directly assessing neuropsychiatric traits in relative of people with ALS. The observation of familial clustering of neuropsychiatric signals in ALS kindreds supports the finding of this project, and other ALS family aggregation studies\textsuperscript{314, 354, 355}, that genetic burden in ALS is associated with many pleiotropic neuropsychiatric manifestations. Though a novel observation, it is somewhat unsurprising that higher levels of psychosis risk were detected among ALS relatives compared with controls, with the former being over three times more likely to exceed pathological cut-offs for high psychosis risk. Dating back to the early 1900’s, there have been case reports of patients with ALS who experienced persecutory ideation and hallucinations during the course of their illness\textsuperscript{546-548}. Indeed, ALS and schizophrenia share many overlapping physical and cognitive features, including prominence of executive dysfunction\textsuperscript{16, 367}. Familial forms of schizophrenia are reported to have an excess of minor physical anomalies\textsuperscript{549}, with neurophysiological studies showing subtle evidence of motor neuron denervation and reinnervation among patients with schizophrenia and their relatives\textsuperscript{548}, and both disorders show evidence of enhanced cortical hyperexcitability, particularly of frontal tracts\textsuperscript{446, 550}. Recent epidemiological\textsuperscript{314, 354} and genetic studies\textsuperscript{315, 551} have provided additional evidence in support of this putative link. Together, this suggests that high psychosis risk among relatives, detected using the CAPE-P15 questionnaire in this study, may have utility as a quantifiable marker of genetic risk. Enriching genetic study cohorts for individuals scoring highly on this measure may enhance their ability to detect genetic variants indicating the involvement of specific biological pathways shared between ALS and psychotic traits.

It is notable also that this study found higher levels of initiation apathy among ALS relatives, particularly those from familial ALS kindreds. This is the same cohort which demonstrated higher levels of phonemic verbal fluency deficits in the previous two chapters. This is of interest as initiation apathy is characteristic of ALS\textsuperscript{472} and has previously been shown to correspond with phonemic verbal fluency deficits in patients\textsuperscript{458, 474}. The replication of this pattern among familial ALS relatives supports the existence of a heritable common trait linking both symptoms. Initiation apathy refers to impairments associated with self-generation of behaviours or cognition, rather than difficulties with attention/planning or emotion integration (executive and emotional apathy respectively)\textsuperscript{472}. Stuss’s model of executive function\textsuperscript{552}, particularly the concept of energization which reflects the ability to initiate and sustain responses to tasks, can be used to link initiation apathy and verbal fluency impairment as a shared process involving
initiation of thought and action respectively. Neuroimaging studies support the overlap with the anterior cingulate gyrus, caudate nucleus and the medial prefrontal cortex all previously implicated in apathy and verbal fluency deficits in ALS. As both verbal fluency deficits and apathy are the most common cognitive and behavioural changes in ALS respectively delineating the nature of this relationship and the extent of its genetic underpinnings will hopefully provide insight into the underlying disease processes.

A similar correlation may be could be made between the lower openness to experience traits, lower IQ and verbal fluency deficits in familial ALS relatives. Openness to experience describes the extent to which an individual accommodates and seeks new information and ideas, with individuals who score highly on this measure showing greater adaptability to novel exposures. People who score highly on openness to experience have a propensity for novelty and learning and as such may acquire greater semantic knowledge and intellectual skills over a lifetime. This observation has been proposed as an explanatory factor underlying the positive correlation between openness to experience and intelligence scores, particularly those based on verbal knowledge. This propensity towards lifelong learning could also explain an association with higher levels of cognitive reserve. Conversely, lower scores on this openness to experience may have important implications, particularly in regard to clinical decision making and genetic counselling, as individuals who score low are more likely to rely on familiar information and be less inclined to consider alternative opinions. C9orf72 negative relatives from C9orf72 kindreds, who arguably are most likely to be required to take up an informal caregiving role, also recorded the lowest scores on openness to experience. Particularly for this cohort, it may particularly important in counselling/providing advice to build on a framework of what they are already familiar with, as an approach for introducing new concepts.

Finally, an interesting observation was noted for the C9orf72 positive relatives who showed the highest levels of self-control when younger, with this rapidly declining across increasing age-cohorts, such that they had the lowest levels of self-control among those aged over 55 years. This could correlate with two phenomena previously reported among carriers. Firstly, disinhibition is one of the most common behavioural changes observed among C9orf72 carriers with FTD and ALSFTD. Conversely, the premorbid lifestyle among asymptomatic C9orf72 carriers suggests high levels of discipline, with higher levels of exercise and lower BMI and alcohol consumption in this cohort. While this study’s finding requires replication in a larger cohort, it suggests that C9orf72 carriers are genetically predisposed towards maintaining a certain lifestyle which may contribute
to decompensation of vulnerable neural networks, resulting in an earlier active disease process manifesting as disinhibition. This is supported by work by Julian et al (2020) who showed that exercise is a risk factor for ALS, specifically among those carrying the C9orf72 repeat expansion. Furthermore, my study’s findings would suggest that there is considerable putative utility in using measures of disinhibition as markers of progression in the pre-symptomatic period.

12.9.1. Limitations

The limitations of this study are shared with those outlined in Chapters 10 and 11. In particular, the sub-categorisation of participants into age-categories in this study likely resulted in a significant loss of power. In this regard, the recruitment of additional age-matched controls would allow for greater examination of the data collected. This would also facilitate an assessment into the extent of correlations between neuropsychiatric variables from this study and neuropsychological data reported in the previous two chapters. Finally, relatives who declined to participate in this study were more likely to have performed poorly on verbal fluency tasks. If this measure is shown to correlate with neuropsychiatric variables suggested above, that would infer that the extent of impairment on these measures (apathy, openness to experience) may be under-estimated in the current study.

12.9.2. Conclusion

The findings of this chapter align with those of the previous two chapters in demonstrating extensive neuropsychiatric dysfunction among relatives of those ALS. This signal clustered in familial ALS kindreds and was not explained by the pathogenic C9orf72 repeat expansion. The particular prominence of these findings among C9orf72 negative relatives from C9orf72 kindreds suggest the existence of other pleiotropic gene variants within these kindreds, which may account for the significant phenotypic heterogeneity among C9orf72 carriers. Particularly, given the prominence of both symptoms in ALS cohorts, examination into the extent of correlation in verbal fluency performance and initiation apathy among relatives is warranted. This ‘energization’ variable could be a putative endophenotype shared between both symptoms which likely offers a more direct means to identify underlying genetic mechanisms.
13. Chapter 13: Summary of Findings and Discussion, Conclusions, Limitations and Future Directions

13.1. Introduction

The primary aim of this project is to comprehensively examine the influence of genetic risk on the phenotypic manifestations of ALS, both for the individual and for their families. Greater insight into the genetic underpinnings of ALS provided by this project will help advance our understanding of the key pathobiological pathways involved and facilitate the identification of potential novel therapeutic targets. This is urgently required for ALS, a disease which is relentlessly progressive, ultimately fatal and without current curative or significant disease ameliorating treatments.

To comprehensively test the primary hypothesis, I defined three key study aims. Firstly, the extent to which genetic risk inherited from distant ancestors influences ALS risk and phenotypic manifestations was examined. Secondly, the significance of familial clustering of ALS and other neuropsychiatric disorders was delineated. Finally, the cognitive and neuropsychiatric profile of relatives of those with ALS, and how this is influenced by known ALS-associated variants, was characterised.

In this chapter, I summarise of the key-findings from the project studies and discuss them relative to existing ALS literature and current practices. Project limitations are highlighted. Finally, I look forward toward the next key steps in this research endeavour, particularly those pertaining to the genetically at-risk population.

13.2. Advancing ALS epidemiology

To start, this project determined, for the first time, that the current lifetime risk of developing ALS for the Irish general population to be 2.9 and 2.3 per 1,000 men and women respectively, corresponding to 1 in 347 men and 1 in 436 women. These estimates were obtain using age-standardised ALS incidences rates and were corrected for competing causes of mortality. The results are very similar to the risk estimates reported for the UK population\(^5\). However, this project expands on previous work by demonstrating first-degree relatives of “gene-negative” ALS patients are at increased risk of developing ALS compared to the general population. This has important genetic counselling implications. Notwithstanding, the possibility that in the future as-of-yet unidentified Mendelian inherited ALS gene variants may be identified in some of these
proband, current concerned relatives are left in a difficult situation where they can be described to be at increased risk, but are without means to perform predictive testing. In this respect, it is worthwhile emphasizing that even though the risk was increased, the absolute risk among these relatives was found to be very low (lifetime risk: relatives 0.7%, general population 0.3%).

Secondly, this project reports that the annual age and sex-standardized ALS incidence in Ireland has remained stable over the last 22 years and is broadly in line with that reported for other European populations\textsuperscript{234}. In spite of this, I also identified an apparent temporal increase in familial ALS incidence over the same time period. Ultimately, this finding directly reflects the many strengths (and a potential pitfall) of long-running population-based disease registries.

The Irish ALS Register is the longest running true population-based register in the world for ALS, and garners data on the predominantly genetically homogenous and relatively demographically stable Irish population. This resource was able to show that with comprehensive case ascertainment, the incidence of familial ALS is close to 20% but may be greater if extended phenotypes are included in this definition. This feat was only achieved through meticulous identification and linkage of the prevalent Irish ALS kindreds, such that currently over 50% of new familial ALS cases are recognised as belonging to a known ALS kindred. The pitfall came in the form of information creep with the increasing recognition of the extended phenotypes within ALS kindreds contributing to the apparent temporal increase in familial ALS incidence. Yet, while considered a bias for data analysis purposes, this finding demonstrates clearly the strengths of a long-running population register in providing insights into the whole phenotypic spectrum associated with shared genetic risk. Detailed interrogation of the comprehensive data accrued in the Irish ALS Register has already provided many insights into disease pathogenesis and heterogeneities of the clinical phenotype in ALS\textsuperscript{486} (including work in this thesis) which might not have been possible using shorter-term or non-population-based Registers.

Prompted by a lack of clarity surrounding whether inclusion of extended ALS-associated phenotypes into the current classification criteria for familial ALS is warranted, I have expanded definitions to encompass kindreds with significant clustering of FTD or schizophrenia based on the convergent findings of two probability models. The younger age of onset among ALS probands from these kindreds, best fit a model of increased shared genetic risk, similar to that proposed for more classical familial ALS presentations\textsuperscript{311}. Furthermore, these findings align with recent work by McHutchison et
al (2020) who showed an association between a family history of neuropsychiatric disorders, particularly mood disorders, and cognitive performance in ALS probands. Both works, while using different approaches, are consistent in demonstrating 1) the impact of a shared neuropsychiatric genetic risk on the phenotypic presentation of ALS probands and 2) that this genetic risk is not entirely attributable to the C9orf72 repeat expansion. While many argue for valid reasons that family history taking is of limited utility in ALS, my work provides hard evidence that close attention to family history is extremely valuable, and may help to decipher the factors underpinning disease heterogeneity. I have shown how methodical and detailed family history taking can advance our understanding of the expanded phenotype associated with ALS. Even, a less detailed, heuristic approach can be used to identify kindreds with significant familial clustering of neuropsychiatric signal which likely harbour important, unidentified, pleiotropic gene variants.

Furthermore, considering a clan genomics model, I investigated variance in phenotypic manifestation in ALS across populations of different ancestral origin. Adding to the literature on ALS epidemiology in understudied populations, I have shown that that Cuban ALS patients developed symptoms approximately nine years earlier than Irish ALS patients. Byrne et al (2013) previously demonstrated that such geographical differences in mean age of ALS onset can be attributable to differences in life expectancies across populations. Greater adversity (poverty, social/trauma, neighbourhood hardship etc) in developing parts of the world may become biologically embedded through the accelerated aging of cells, tissues, and organs and contributing to lower life expectancies in these regions. In this model, the younger age of ALS onset would simply reflect the underlying population age structure. This is supported by work by Zhang et al (2020) who demonstrated that greater DNA methylation age acceleration was associated with a younger age of ALS onset. Detailed age/period/cohort studies of large and diverse populations would be helpful further explore this hypothesis.

With respect to the comparative analysis across Cuban, Uruguayan and Irish clinic-based populations conducted for this project, equal life expectancies were reported across the different populations at the mid-point of this study. Similar life-expectancies were observed for the other Hispanic/Latino clinic-based populations studies reviewed in respect to age of onset and corresponding survival periods. This apparent discrepancy is in keeping with historical patterns reported by Turner et al (2012) who noted that increases in the mean age of onset of ALS lagged behind improvements in life-
expectancies in some populations, sometimes by decades. This suggests that the relevant ALS risk exposures may occur early in life and as such may not be mitigated by later socioeconomic advances. This is supported by work by Marini et al (2020) who found that impact of adversity on perpetuating altered methylation processes may be heightened during specific life stages, particularly early childhood. As such, a decline in population-level exposures or behavioural patterns relevant to these life stages (e.g., smoking during pregnancy which may induce both somatic mutations and epigenetic effects) may not impact on population-level disease liability until many decades later.

Beyond shifting population demographics, the younger onset of disease among Cuban ALS patients could also reflect greater exposure to one or more risk factors. In this regard, it is interesting that the literature reports on two apparently distinct cohorts arising within the younger ALS population. Firstly, an earlier mean age of onset is observed among familial ALS cases, with familial cases estimated to require less additional steps than sporadic cases on the multistep model of ALS development. This was shown by Mehta et al (2019) to be attributable to the presence of Mendelian-inherited gene variants, rather than reflect ascertainment bias. These pathogenic genes, including the pathogenic C9orf72 repeat expansion may induce more rapid age acceleration with DNA methylation driving earlier disease onset and shorter survival.

By contrast, a second cohort of young onset ALS patients may be observed characterised by male prevalence, spinal onset, upper motor neuron predominant phenotype, slower disease progression and longer survival compared with older ALS cohorts. It is interesting to note that the gender difference was only observed for young-onset ALS patients with an upper motor neuron predominant phenotype. Young onset cases with a more classical presentation showed near equal gender mix. This male upper motor neuron pre-dominant cohort likely lies on a clinical and pathological continuum with PLS, a disorder which equally shows male predominance and younger disease onset, and in whom genetic causes are also not identified in the majority of cases.

While no significant difference in other phenotypic variables were detected across population or within Cuban population groups, the Cuban ‘mixed ancestral’ group were the youngest, with slight male and spinal onset predominance. This group was previously shown in a population-based mortality by Zaldivar et al (2009) to have lower ALS rates compared with those of Spanish ancestral origin. Interestingly, in this project’s work, only 24% of ALS diagnoses in the Cuban study cohort occurred in the admixed “mulatto” population and 12% in the “black” population, despite equal access to healthcare across
all ethnicities in Cuba. While the incidence in the “black” population reflects the underlying Cuban population composition (55% “white”, 33% “mulatto”, 12% “black”)\textsuperscript{324}, ALS incidence rates in my study were higher in white population and lower in admixed populations than should be expected\textsuperscript{324, 567}. These findings mirror that of the cohort distribution of the US National Longitudinal Mortality Study\textsuperscript{568} where only 24.4% of all ALS cases diagnosed where of not of “White, non-Hispanic” origin (“Black, Non-Hispanic 9.4%, “Hispanic” 10.3% and “Other Ethnicities, non-Hispanic” 4.7%). The higher rate of ALS among whites compared with other ethnicities was not found to be a result of ascertainment bias due to variance in socioeconomic status, birthplace, and access to health insurance across the different groups but more likely reflects true increased risk in this cohort\textsuperscript{568}. Differing ALS risk and phenotypic manifestations in these populations may arise as a direct effect of genetic admixture.

Genetic admixture is hypothesized to be protective as regards to ALS risk, through reducing the exposure to potential risk alleles. This is supported in part through the identification in this project of the unique genetic signature within the Cuban population with respect to known ALS variants. The Cuban population had a low prevalence of C9orf72 repeat expansion carriers and no carriers of any known SOD1, TARDBP or FUS mutations were identified, highlighting the diminished importance of these variants in this population. By contrast, adaptative introgression may occur in admixed populations, with increases in introgressed haplotypes reflecting a selective evolutionary effect. Neurodegenerative diseases typically affect individuals after their reproductive years, and so there is a low selective pressure to eliminate perilous alleles. Norris et al (2018)\textsuperscript{323} found evidence of adaptive introgression in the admixed Latin-American genome, using ancestry-enrichment analysis to identify locus-specific haplotype loci (locus-specific ancestry patterns not accounted for by the overall ancestral profile of the population). Ancestry-enriched SNP genes were implicated in a number of pathways (immune system, metabolic, signalling pathways) and disease phenotypes (type 1 diabetes mellitus, Alzheimer’s disease). While much more work is required to explore the impact of increasing genetic diversity on mitigating ALS risk, these ancestry-enriched genetic patterns may also introduce their own risk profile, with potential implications for disease pathogenesis and future treatment.

13.3. **Sex-specific disease liability**

This work highlights the importance of population-specific genetic signatures. In this project, I conducted the largest population-based study on ALS heritability to date. An important novel finding of this study, was the observation of higher heritability estimates
for female patients strongly supporting the existence of a sex-mediated effect on ALS liability. This finding propelled subsequent work by Byrne (2021) who identified that a significant portion of ALS SNP-based heritability is explained by sex-interactions, with lower SNP-heritability estimates present in male patients. Byrne (2021) identified a number of novel and known variants contributed to sex-mediated differences in ALS risk, conferring different magnitudes of effects in each sex, or alternatively not prompting an effect in one sex. Several of these sex-specific loci are highly expressed in the CNS, with many implicated in the processes of neuronal growth and morphogenesis. Finally, Byrne (2021) reported that while the majority of genetic effects are likely shared across sexes, genetic risk in ALS is explained by a much smaller set of variants in males than in females. This implies that females require more risk alleles to develop ALS (higher polygenic burden). This, in turn, confirms the higher ALS heritability estimates for the female sex.

As discussed previously, the higher heritability estimates in females can be considered in relation to the threshold of liability model. Using this approach, affected females are expected to deviate more from the mean of their sex than affected males do (as the incidence of ALS in general population is lower for females). If the disease liability is considered to stem from genetic factors, affected females would be more likely to transmit a higher genetic risk profile to their offspring. Indeed, this sex-differentiated risk of transmission patterns, was observed in a study by Fang et al (2009) where children whose mothers had ALS had a higher risk of developing ALS compared with children whose fathers had ALS. This phenomenon of a lower disease incidence but higher rates of transmission for one sex is coined the ‘Carter effect’, after Cedric Carter who first observed that females with pyloric stenosis were more likely to transmit the disorder to their children, despite a lower prevalence of the disorder among women. Under this model, the relative risk of developing a given disorder is also expected to be greater among siblings of female probands compared with siblings of male probands. With regard to ALS, this proposition is supported indirectly in the observation by Gibson et al (2014) who noted a higher risk of ALS among maternal rather than paternal uncles in their familial clustering study. This higher relative risk has also been observed in siblings of female probands for other neurodevelopmental or neuropsychiatric disorders, where a higher disease incidence is reported for males.

Carter’s original sex-dependent liability threshold model considered that females were protected by some factors against developing pyloric stenosis, explaining the lower female specific-incidence in the general population. Consequently, those women who
became affected with the disorder must be considered to have a greater genetic liability to overcome these protective effects. It is arguable that such a model would also fit with female-specific ALS liability, with female ALS patients requiring a greater genetic burden to overcome potential ALS protective effects. Several authors have reported on the protective effect of female sex-hormones, with both endogenous and exogenous hormones shown to ameliorate ALS risk\textsuperscript{494, 495}. Whether post-menopausal treatment with hormone-replacement therapy offers some neuroprotective benefit with respect to ALS is unclear. Popat et al (2006)\textsuperscript{575} found no benefit to treatment in a small US study, contrasting with the findings of Rooney et al (2017)\textsuperscript{495} who observed a strong protective effect only for Netherlands cohort in a EURO-MOTOR group study. Lower prevalence of HRT users at the other EURO-MOTOR sites may explain the lack of treatment effects at those sites. The study authors also highlighting the centrality of timing and formulation of exogenous hormone treatment, referencing the “timing hypothesis” where the neuroprotective effect is only seen if treatment is initiated within a critical treatment window peri-menopause\textsuperscript{576, 577}.

If Carter’s model infers that women who develop ALS carry a greater genetic burden, then conversely environmental exposures must play a greater role in disease development among male ALS patients. This aligns with the lower male sex-specific heritability estimates observed in this study, and with the findings by Byrne (2021)\textsuperscript{551} who noted a higher proportion of non-genetic confounding in their male-specific GWAS. These non-genetic factors increasing ALS liability could be endogenous. For example, higher prenatal testosterone levels are purported to increase the risk of developing the disorder as evidenced by lower index-to-ring finger length ratios observed among sporadic ALS patients\textsuperscript{578}. Alternatively, exogenous environmental exposures may preferentially increase ALS risk in males. For example, data from OECD\textsuperscript{579} shows that males dominate many industries most strongly linked to ALS including agriculture, fishing, mining, construction, metal work and mechanics with male: female ratios in these industries ranging between 15-80: 1. Of note, many other putative risk factors also linked to ALS are shared across these industries, including exposure to heavy metals, pesticides, chemicals, dust irritation, air pollution, extremely low-frequency electromagnetic fields and requirements for intense physical activity. \textsuperscript{223}.

In reviewing how environmental exposures could play a greater role in ALS development among males, it is worth reflecting on the order of causality. In ALS, putative lifestyle factors (e.g., fitness phenotype) are believed to result from a common genetic profile conferring increased ALS susceptibility\textsuperscript{294, 306}. It is possible that this may also be the case
for factors such as occupation, although undoubtedly societal norms play an influential role also. In this model, genetic burden drives certain personality traits/lifestyle choices which ultimately results in the development of the disease. However, this model does not explain the pattern of increased maternal transmission or the lower heritability estimates for males. It is worth recalling the term heritability describes only the proportion in the variance in ALS risk which is attributable to genetic factors, not the totality of the disease liability. Rather, heritability could be better considered in terms of whether genetic or environmental factors have greater influence on pushing one past the threshold of disease liability. For males, it appears that environmental factors likely play a more significant role in ALS pathogenesis that in females.

Of course, some males carry pathogenic ALS mutations. This cohort could be considered to represent a “double hit” scenario, as they presumably carry high burdens of both genetic and environmental risk. As such, these patients would be expected to have a more severe phenotypic expression. Indeed, work by Rooney et al (2016) would support this hypothesis as the authors demonstrated that male ALS patients who carried the C9orf72 repeat expansion were younger at onset and had a worse prognosis than female carriers. They were also more likely to have experienced a shorter diagnostic delay, suggesting a more rapidly progressive disease course. Other genes may act as modifiers of phenotypic expression in C9orf72 positive males, as evidenced by the moderate heritability estimates for C9orf72 negative male-specific pairings. Yet, it is probable that for males environmental factors likely play a greater role in phenotype modification than for females as sex-specific C9orf72 negative heritability estimates are much higher in the latter.

This leads onto another interesting concept, which is that women with known pathogenic ALS genetic mutations likely require additional genetic risk to develop ALS so as to overcome any potential protective environmental factors. This would mean oligogenic inheritance patterns are more likely to be observed among age-matched female patient cohorts. This could explain, in part, the findings by Van Blitterswijk et al (2014), that intermediate ATXN2 expansions act as disease modifiers among C9orf72 repeat expansion carriers, rendering carriers of both expansions more susceptible to the development of MND as opposed to FTD. Subsequent work by Rubino et al (2019) identified that intermediate ATXN2 repeat expansions may modify the clinical phenotype among those with FTD. In fact, the former study’s findings may simply stem from the failure to detect any intermediate ATXN2 expansions in the C9orf72 FTD cohort, which can be explained by the low hit rate of the intermediate ATXN2 expansion (4/266 C9orf72 expansions).
carriers), the small FTD cohort size and the lower proportion of females in this cohort compared to the C9orf72 positive MND cohort (FTD [40%], MND [51%]). Further work, however, is required to examine the influence of sex on phenotypic manifestations arising from oligogenic inheritance patterns.

Finally, this sex-dependent liability model would also suggest that a higher proportion of females border the threshold of ALS development, but have not crossed it. These females may have a high genetic risk burden but are either 1) protected from developing the disease by other factors or 2) they may not have inherited the required oligogenic variants necessary to propel the disease. This would manifest as delayed disease onset or reduced penetrance patterns among females and is supported, in part, by observation by Murphy et al (2017)\textsuperscript{240} that penetrance is reduced among female C9orf72 carriers compared to male carriers across the entire age range. Furthermore, this model suggests that if a forme fruste of ALS exists, it would be more likely observed among females. A forme fruste variant of any disease can be considered in relation to the threshold of liability model to represent individuals who have sub-threshold clinical symptoms (Figure 13-1). They are considered as phenotypic manifestations of a high genetic risk. However, this risk is not great enough, or they have had the relevant environmental exposure to cause the full disease manifestations. If endophenotypes are quantitative traits reflecting genetic liability, forme fruste would represent a greater degree of abnormality for the measure e.g., psychosis risk exceeding pathological cut-offs.
Endophenotypes have been used in the field of psychiatry for over four decades, with more recent applications in the neurology world. They offer a simpler means of considering complex clinical disorders as a composite of multiple vulnerability traits reflective of genetic risk. While identification of endophenotypes for specific disorders in many cases has remained elusive, putative endophenotypes have already assisted in the identification of several novel pathogenic mutations. For example, the use of electrophysiological endophenotypes in addition to clinical diagnoses were used in a series of studies by the Collaborative Study of the Genetics of Alcoholism (COGA) to identify GABRA2 and CHRM2 as variants associated with predisposition to alcohol dependence. More recently, large GWAS studies of cognitive endophenotypes in schizophrenia have identified variants including ATXN7, CSMD1, DRD2, GRIN2A, GRIN3A, and GRM3 and highlighted functional gene networks impacting on neuregulin and glutamate pathways. Identification of endophenotypes may also facilitate
the study of functional consequences of risk alleles, providing insight into disease mechanisms.

For example, a recent GWAS implicated RIMS1 variants in semantic verbal fluency which have previously been linked to cognitive performance and schizophrenia. This cognitive endophenotype showed the greatest differences in relative-control performance in a meta-analysis of endophenotypes in schizophrenia, with a tendency to cluster in familial schizophrenia kindreds. Furthermore, these deficits have been reported consistently across schizophrenia populations with different language systems and cultural backgrounds. In contrast to phonemic verbal fluency deficits which show greater prominence in ALS patients and among ALS relatives in this project, semantic fluency deficits in schizophrenia induce greater activation in left temporal cortex and posterior cingulate, reflecting more activation of episodic retrieval processes. However, both fluency paradigms rely heavily frontal-striatal connections, evidencing the significant neural network overlap between ALS and schizophrenia.

This is supported by a recent GWAS study by Byrne (2021) identified several putative pleiotropic loci linked to ALS and psychiatric traits, including CNNM2, SRGAP1, KRT18P55, SRGAP1, NCKAP5L, SPIRE1, AS3MT and C9orf72. This study supports the epidemiological findings of this project which suggest that not all psychiatric manifestations in ALS should be attributed to the C9orf72 repeat expansion. Furthermore, Byrne (2021) found strong evidence of a lack of genetic overlap between ALS and depression, again supporting the epidemiological findings of this project and supporting the hypothesis that inconsistently reported higher rates of depression in ALS families likely reflect a reactive rather than a direct causal association. Finally, identification of genetic variants associated with ALS risk is necessary to enable a precision medicine approach to treatment. The sex-specific gene variants identified by Byrne (2021) may have important clinical implications for clinical trial design. UNC13A was found to demonstrate stronger evidence of association with ALS among females. This mutation was shown to strongly influence the treatment effects (survival) of lithium carbonate in ALS patients, highlighting the importance of consideration of sex in developing targeted precision medicine treatments in ALS.

13.4. ‘Development Risk Factor Model of ALS’

It has become increasingly clear that a reductionist neurodegenerative or neurodevelopmental disorder model will never fully account for the pathogenesis of ALS. Evidence of the presence of structural, functional and clinical changes observable
decades before overt symptom development in pathogenic ALS gene mutation carriers argues against the former, with the late-onset nature of the disease arguing against the latter. Instead, I propose that the model that best fits ALS is the 'Development Risk Factor Model' which is already applied to disorders such as schizophrenia. This model postulates that, in ALS, genetic factors or early environmental exposures are the foundation for the development of anomalous neural networks. These vulnerable neural networks are evidenced by the widespread sub-clinical cognitive and neuropsychiatric changes detected among relatives of people with ALS. The phenotypic outcome of this risk profile may be modified over a lifetime through additional environmental exposures, with the interaction with some factors e.g., physical exercise potentially causing diverging phenotypic effects (protective: neurocognition, psychosis; detrimental: motor symptoms). An accumulation of toxic exposures/lifestyle, or perhaps just aging alone, eventually forces a decompensation in these vulnerable networks with the development of overt symptomology and a self-perpetuating disease process.

In support of the 'Developmental Risk Factor Model' is the multitude of evidence supporting a genetic basis for ALS. In this project, just over 50% of the variation in ALS risk was found to be attributable to genetic factors. While rare variants are believed to play a central role ALS pathogenesis, the shared polygenic risk between ALS and other neuropsychiatric disorders is likely linked to changes in cognitive development through impaired function of essential neurodevelopment processes. The GWAS study by Byrne (2021) expanded on the previous findings of their group showing significant polygenic overlap between ALS and schizophrenia, by verifying and extending this finding to include bipolar affective disorder and cognitive impairment. Through latent causal variable analysis, Byrne et al (2021), using data from our cognitive group, showed that ALS genetic burden is causally linked to lower cognitive performance, but not to schizophrenia or bipolar affective disorder. Regarding the former, this has significant clinical implications as it would infer that potential treatments for ALS may not only stall motor progression but also cognitive decline. The latter observation suggests that while ALS and other neuropsychiatric disorders may share some overlapping biological pathways, they also feature disease specific mechanisms.

Genetic susceptibility may be further impacted by environmental insults during sensitive periods in brain development. Brain development can be considered as the evolving processes of neurogenesis, neuronal migration, gliogenesis, synaptogenesis, myelination and synaptic pruning, spanning over two decades, commencing in utero. Insults during particular time sensitive windows may result in long-lasting disruption in
brain circuitry organisation. For example, visual deprivation in the early developmental period has been shown to result in disruption of cortical connections and aberrant neuroanatomical development. In schizophrenia, peri-natal insults including maternal malnutrition, prenatal infection and peri-natal injuries have all been associated with an increased risk of the disorder, although it may be noted that some apparent peri-natal insults may reflect genetic loading rather than direct teratogenic effects (e.g., maternal smoking during pregnancy and mental illness in offspring). Nonetheless, whether through environmental or genetic means, the disturbance of neural network development during these time sensitive periods is the foundation of the development of schizophrenia.

Recent structural and functional evidence supports that similar neural network vulnerabilities play a central role in pathogenesis of ALS and other neurodegenerative disorders. In patients with ALS, neurophysiological approaches have shown widespread disease-associated network disruption of motor and cognitive networks, correlating with clinical measures. For example, measures of disinhibition have been linked to differentiated heightened activity in the left frontoparietal network, with decreased signalling in bilateral frontal regions. Similar dysfunction of prefrontal and temporal networks has been observed in FTD patient cohorts. Such neurophysiological markers of neural network dysfunction, when combined with clinical information have been shown to improve diagnostic accuracy. Pre-symptomatic studies in both FTD and ALS cohorts have provided evidence of structural network change over two decades before the development of overt clinical symptoms with evidence of functional network change present up to three decades before symptom onset for FTD gene carriers. While functional studies for pre-symptomatic ALS cohorts are lacking, recent interest in this area has provided some preliminary evidence of neurophysiological changes in asymptomatic C9orf72 repeat expansion carriers, although the numbers of carriers yet studied is very small.

Toxic insults during time sensitive periods have previously been shown to alter neural network developmental patterns, with subsequent neurological decline occurring many decades later in the case of poliomyelitis virus and post-polio syndrome. Differential neural reorganization patterns were seen between children who were affected by polio under two years of years and those who were exposed later in childhood. As mentioned above, many putative environmental risk factors for schizophrenia occur peri-natally or during infancy. Yet, the importance of timing of environmental exposures in ALS is less clear, largely due to the much longer time periods for potential exposures for most
patients. However, for the young onset sporadic ALS cohort, it is probable that the timing of any environmental exposure plays a critical role. A toxic insult in a critical time period could markedly reduce the number of additional steps required for ALS development (multistep model\(^\text{\textsuperscript{603}}\)), similar to what has been observed for large effect gene variants\(^\text{\textsuperscript{310}}\).

Alternatively, in a susceptible population with genetically derived vulnerable neural networks, cumulative toxic exposures over a lifetime may be required for ALS development or may alter its phenotypic manifestations. For example, vulnerable networks may manifest certain personality or cognitive traits (less open to experience, lower IQ) which may drive a cycle of reduced engagement with learning activities over a lifetime, resulting in lower cognitive reserve and a greater likelihood of a cognitive onset presentation for ALS. Carriers of high-risk genetic mutations (e.g., C9orf72 repeat expansion carriers) may be predisposed to maintaining certain lifestyle choices (e.g., physical exercise) which in turn may be associated with younger onset of disease (‘double hit hypothesis’). Indeed, asymptomatic C9orf72 carriers have been shown to be preferentially predisposed to exercise-induced ALS\(^\text{\textsuperscript{558}}\). However, at present, there is no evidence to support any primary preventative measure, even in targeted cohorts and doing so could potentially do harm (e.g., reducing exercise/increasing could result in increased cardiovascular risk profile). Even the identification of this at-risk population is problematic, as the vast majority of people who develop ALS have no family history of the disorder and do not carry any known large-effect pathogenic mutation. However, in the future, this problem may be solved with combinations of functional or other biomarkers (the latter preferred because of low cost and non-invasive nature) and neuropsychological measures helping identify those with evidence of abnormal neural network construction. In light of the extensive cognitive and neuropsychiatric changes evident among relatives of people with ALS, this population may be most informative in developing these measures for research, while equally having potentially having the most to benefit from study participation.

13.5. Conclusion

Much of the work of this project can be directly applied in an ALS genetic counselling setting. The clan genomics model provides a strong framework for conceptualized both ancestral and recent family inherited genetic risk. Symptomatic and asymptomatic individuals can be counselled that 50% of variability in ALS risk within a population is accounted for by genetic factors. While this does not pertain to individual risk specifically, it would suggest that genetic and environmental factors play approximately equal roles in the development of ALS. Furthermore, in the absence of known pathogenic mutation
running in their kindred, first-degree relatives of people with ALS can be told that while their risk is slightly elevated compared with the general population, the risk of them developing ALS remains less than 1%.

My work has focused on familial ALS. Recent advances in our knowledge of the shared genetic risk between ALS and other neuropsychiatric disorders prompted a re-evaluation in what we define as significant familial clustering of disease. This shared genetic liability is further underlined by the extent of overlap in neural network dysfunction, evidenced through the identification of novel putative cognitive ALS endophenotypes. The family context may play an epigenetic important role, particularly during specific life-stages. Familial clustering of disease may occur due sharing of other non-genetic factors (e.g., educational attainment, occupation) or may be mediated through gene-environment interactions.

While a health care professional's primary duty of care is to their patient, oftentimes, they will strive to provide support also for family members of patients, as appropriate. This may be through direct (practical/psychological support) or indirect (policy implementation, advocacy) means. In ALS and many other disorders, family members provide the bulk of informal caregiving and play a central role in clinical decision making. In turn, they face tremendous burden, psychological distress and reduced quality of life. In this context and in light of the evident shared genetic risk, psychosocial exposures and life experiences, it is arguable that the unit of care should broaden from that of an individual patient to a more holistic approach encompassing the family unit.

A patient- and family-centred care (PFCC) approach has been suggested as a means of providing contextualised care, taking into consideration the patient’s broader life experiences. While maintaining the centrality of patient autonomy, it also recognises the importance of the patient’s family as essential partners in improving care practices. By implementing PFCC principles of active listening to family, facilitating choice, sharing information and building confidence, PFCC has been shown to improve patient outcomes, decrease healthcare utilization and costs and increase patient and staff satisfaction. The central components of this family centered care model includes 1) collaboration between family members and health care providers, 2) education for patients, families, and healthcare providers, 3) consideration of family contexts and 4) dedicated policies and procedures (Figure 13-2). In the field of neurology, this approach has so far been adopted primarily by the paediatric services, including in the care of children with cerebral palsy and childhood epilepsies. By contrast, very little has
been done with this model with respect to neurodegenerative disorders. A systematic review by Hao et al (2020)\textsuperscript{612} identified only nine small studies looking at family centeredness in dementia. Though the outcomes of these studies were predominantly positive, the family centered interventions varied greatly in design and health setting necessitating further work to delineate best practices in this area.

![Figure 13-2: Family Centered Care Model.](From Kokorelias et al (2019)\textsuperscript{613})

Consideration of the family unit is arguably essential in ALS for three reasons. Firstly, a central component of this project has focused on the shared genetic risk and associated phenotypic manifestations within families. With regards to disseminating this genetic risk information within a kindred, current policies place the proband in the position of gatekeeper\textsuperscript{614}. As such, they become responsible for striking the appropriate balance between sharing important information while maintaining personal privacy and protecting family members from undue distress or harm. However, due to the inherent shared nature of genetic risk, recent proposals have suggested that genetic testing and counselling should be treated as a family process from the outset\textsuperscript{615}. While this approach
is believed to have a positive impact on how some families adapt to genetic threat, there are some inherent limitations. For some individuals this approach may feel like an invasion of privacy. Others may feel pressurised to proceed with genetic testing or it may cause strain within some family relationships. Nonetheless, should current policies change to allow for a greater family-centered approach to sharing genetic information in the future, familial genetic discussions would need to focus on 1) disease specific measures, 2) individual adaptation processes pertaining to genetic risk and 3) family specific context and variables.

Secondly, we have seen in this project the extensive cognitive and neuropsychiatric manifestation present in relatives of people with ALS. In this context, there are certain clinical issues to consider in relation to care for people with ALS. Patients with ALS often share decision making with family members, particularly when confronted with complex choices. However, given that cognitive impairment is core feature of some forms of ALS, those family caregivers are often expected to take on a greater role in the decision-making process. While more research is recommended in how best to include ALS patients with cognitive impairment in these decisions, as far as I am aware, no research has yet looked at how subtle cognitive changes in relatives may impact on the decision-making process. Similar questions arise pertaining to how asymptomatic relatives’ understanding of the complexities surrounding genetic liability in kindreds may be influenced by their cognitive abilities. In this respect a family centered approach may help health-care teams identify the information and support needs of each unique family and target interventions to improve their knowledge, enhance their self-efficacy and reduce unnecessary stress or harm.

Finally, high risk family units may play an essential role in future research teasing out the risk architecture of ALS. These families can inform us on how both genetic and environmental risk factors interact contributing to specific neural network vulnerabilities resulting in spectrum of phenotypic manifestations within kindreds and as such, can contribute to the development of biomarker profiles indicating dysregulation in specific neural networks. Ultimately, this should lead to the development of a new taxonomy, where ALS and associated disorders can be labelled more aptly into precise constellations of aberrant neural pathophysiology. This approach would facilitate the implementation of timely and personalised interventions which will hopefully lead to substantial improvements in outcome. In the interim, we can strive to develop policies to optimise support for families, both as caregivers for our current patients, but also as a vulnerable population in their own right.
13.6. Project Limitations

This project has comprehensively studied genetic risk in ALS, using a variety of study designs. Several of the project studies were the largest such studies conducted to date (‘ALS Heritability’; ‘Pooled Family Aggregation Study’; ‘Cognitive and Neuropsychiatric Endophenotypes’). Novel and detailed phenotypic and genetic characterisation was conducted in cohorts that previously been neglected (‘Cuban, Uruguayan and Irish Clinic-based Populations’; ‘Cognitive and Neuropsychiatric Endophenotypes’). Furthermore, novel approaches for defining familial ALS were proposed (‘Familial Modelling in ALS’). Finally, this project was strengthened by availability of data from the Irish ALS Register which allowed for direct calculation of ALS incidence and lifetime risk in the Irish population (‘ALS Heritability’; ‘Temporal Trends in Familial ALS Incidence'; ‘Familial Modelling in ALS’). Nonetheless, despite these strengths, several limitations for the project were identified.

Foremost, with respect to sample size across the various studies, there are important limitations to highlight. Firstly, this project targeted recruitment of a minimum of 210 relatives of ALS patients and 100 healthy controls for the ‘Cognitive and Neuropsychiatric Endophenotype’ studies. This goal was achieved; however, a greater number of familial compared with sporadic ALS relatives were recruited. This is likely an artefactual occurrence as relatives from familial ALS families could be considered to have stronger motive to participate such research. Yet, this did not bear true in the observation that not all relatives or controls participated in all aspects of the project. Fewer familial ALS relatives, C9orf72 positive relatives and participants who performed poorly on ECAS ALS non-specific measures went on to complete the detailed neuropsychological assessment. Furthermore, participants who performed poorly on verbal fluency tasks were less likely to partake in the online neuropsychiatric assessment. This suggests that a potential bias may be shared across the project studies, in that relatives with greater cognitive difficulties or sub-clinical psychiatric distress may be less inclined to participate in research. Of course, it can be argued that such biases may also arise when recruiting healthy controls from the general population and, as such, studies may be considered balanced in this regard. Regardless, this would still imply that the true extent of cognitive and neuropsychiatric changes which occur in ALS relatives may be underestimated in this project.

A low number of participants were obtained for some sub-groups within this project’s studies. For example, despite obtaining data from the longest running single disease register in the world (Irish ALS Register est. 1994), only 41 ALS families with significant
clustering of schizophrenia and 18 ALS families with significant clustering of FTD were identified in the ‘Modelling Familial Clustering’ study. While the latter may be artificially low as families with multiple relatives with ALS+/-FTD were excluded from this category, the low number of ALS and schizophrenia families likely results from a failure to recognise the significance of such familial clustering in the earlier years of the register.

Across all studies, one sub-group with consistently low numbers was that of pathogenic C9orf72 repeat expansion carriers (patients and relative sub-groups). While this mutation is the only significant genetically identifiable cause of ALS in the Irish population\textsuperscript{145, 264} it is still a rare mutation with an estimated prevalence of the repeat expansion among healthy adults in the UK of just 0.15\%\textsuperscript{266}. Furthermore, in this project exclusion criteria disallowed anyone with 1) symptoms or signs of ALS or FTD or 2) any neurologic, psychiatric or medical conditions affecting cognitive or neuropsychiatric status from study participation, meaning recruitment of an older but otherwise well C9orf72 carrier population was difficult. Even among those who met inclusion criteria, over 80\% of C9orf72 positive relatives aged between 55-64 years were cognitively abnormal when pathological cut-off values were applied, reflecting insidious pre-symptomatic cognitive decline in this cohort.

In this project, I identified that over 50\% of the variation in ALS risk is likely attributable to genetic factors. Contrariwise, it may be considered that the remaining risk burden is modulated by environmental exposures. Where possible, attempts were made across studies to control for the potential impact of such environmental exposures on study outcomes. In the ‘ALS Heritability’ study, while the impact of environmental variables was not directly assessed, a review of temporal trends in putative environmental risk factors was performed\textsuperscript{233}. Other than smoking habits\textsuperscript{496}, no clear change in these variables over time was identified, which was supported by the observation of stability of ALS incidence within the Irish population over the study period. For the ‘Cognitive and Neuropsychological Endophenotype’ study, participants were asked about potential environmental exposures including occupation, history of head trauma, exposure to heavy metals and cardiovascular risk factors, with no discernible differences detected between environmental risk profiles in relative and control cohorts. Nonetheless, the potential impact of any environmental risk factor may have eluded detection as comprehensive study of such environmental exposures requires highly detailed, prospective longitudinal follow up over a significant time period in large number of people with shared genetic backgrounds\textsuperscript{139}. Such work is undoubtedly important, but costly, time-consuming and beyond the scope of this project’s aims.
As discussed in Chapter 1, putative ALS environmental risk exposures often exhibit strong co-variance, making it difficult to determine the relative contribution to disease risk of each individual exposure. For example, those whose occupations lie in the agricultural, fishing, construction and military industries may be exposed to variety of heavy metals, pesticides, chemicals and air pollution and many such occupations require intense levels of physical activity. In this respect, aggregation of disease within families may arise due to sociological reasons, as occupations may cluster within families e.g., inheritance of family business. Equally, other lifestyle behaviours may aggregate within families influencing disease risk. For example, higher obesity levels in young children have been shown to cluster in families with more irregular mealtimes and high levels of screen time activity in both parents. Such lifestyle behaviours may interact on a high genetic risk background to drive a high prevalence of disease within families. Indeed, identifying such gene-environment interactions in ALS offers potential as a means to intervene and reduce risk in an otherwise high-risk population (ALS gene mutation carriers).

Yet, this is only feasible for cases where known ALS genetic risk profile is completely elucidated. In this regard, a further limitation of this project is the impossibility of controlling for rare ALS-associated variants of large effect which have not yet been identified. Indeed, a key purpose of this project has been to assist in identifying ALS families most likely to carry such variants. This has been achieved through building probability models of familial clustering and discerning which clinical characteristics cluster in familial ALS relatives but are not explained by known genes. Still, until such point that such genetic variants are identified and our ability to understand and delineate their gene-environment interactions remains limited.

This project consisted of several studies which employed diverse study designs, each with specific limitations. Both the ‘ALS Heritability’ study and the ‘Modelling Familial Clustering’ study required the use of lifetime risk estimates in their calculations. Such estimates describe the current risk of developing a disorder for individuals of a certain age, but should not be considered as necessarily directly reflecting the risk for previous or future generations. ‘The Pooled Family Aggregation Study’ 2008-2017 was the largest such analysis looking at neurodegenerative and neuropsychiatric risk in relatives of people with ALS. While data was collected on over 18,000 relatives, this data was obtained via proxy-report by the proband. While the alternative family study method is purported to offer enhanced accuracy of data, comparable results with respect to the sensitivity and specificity of diagnoses in relatives can be achieved with the family history method utilised in this study. Furthermore, recruitment of an equal number of relatives
using the family study method would be prohibitively labour intensive and costly. Finally, the findings the pooled analysis were partially verified by those of the ‘Cognitive and Neuropsychiatric Endophenotype’ study in which relatives of people with ALS were directly assessed using detailed neuropsychological and neuropsychiatric batteries. The latter study was limited in its cross-sectional design. Comparisons across age-categories hinted at declining performance across cognitive measures in relatives of those with familial ALS. A longitudinal study design with sufficient follow up durations would be required to verify these findings. In the regard, the cohort of relatives recruited for this were predominantly young and would provide a good starting point for such a study.

Finally, the study of ‘Clinical and genetic features of ALS across Cuban, Uruguayan and Irish clinic-based populations’ was limited by data from clinic-based series rather than direct population-based measures. Furthermore, interpretation of the findings was limited by poor capture of respiratory and cognitive onset ALS in overall study cohort, absence of genetic data on the Uruguayan ALS population and the inability to comment on the degree of genetic admixture with the available Cuban DNA samples. These issues are addressed in the larger, multi-centre, population-based genome-phenotype correlation studies, conducted as part of the Latin-American Epidemiological Network of ALS (LAENALS) which aims to comprehensively characterise ALS phenotypes in these regions, with capture of relevant environmental exposures. Genome phenotype correlations will be optimised through use of ancestral markers alongside self-reported ethnicity.

13.7. Future directions

In this project, I conducted the largest population-based study of ALS heritability conducted to date and demonstrated for the first-time higher heritability among women with ALS. While estimates obtained in this study are broadly in with those proposed by other authors suggesting generalizability of the findings, nonetheless the findings from this project require replication in other populations. In particular, estimates for populations from Asia, South America and Africa would of interest, given that these regions are expected to experience the greatest increase in ALS prevalence in the next quarter-century. Additional work is required to delineate the genetic factors driving the higher heritability estimates in women. Equally, investigation into the extent to which heritability is shared between ALS and neuropsychiatric disorders would be worthwhile.

In this project, several cohorts of ALS patients have been identified with high genetic risk profiles. These include Cuban ALS patients from familial ALS cohorts, in whom no
currently known ALS-causative genetic mutation was identified. Equally, no genetic cause has yet been identified in the vast majority of probands from families with significant clustering of ALS and schizophrenia. In the future, it is hoped that that the putative cognitive endophenotypes described above may help identify others at high genetic risk. However, at present, only samples from the first two cohorts have been prioritised with our genetics team for more detailed genetic analyses.

In assessing relatives of people with ALS, the age of the participant was used as a proxy to assess changes in performance over time. By contrast, multi-centre FTD groups have largely applied an alternative model, based on time to expected disease onset, with this variable defined by the mean age of symptom onset within a kindred. Using this approach, they have shown the emergence of milder cognitive symptoms in the years preceding FTD diagnosis in known gene carriers. A corollary model is not easily applied in ALS. The age of onset in this context relates to the age at onset of motor symptoms, rather than cognitive or neuropsychiatric symptoms, and at present, how cognitive and behavioural changes relate to ALS stages has not been fully determined.

In the first instance, work is required to determine if the age one develops ALS is predictive of the age one’s relatives will develop ALS. Secondly, to what extent cognitive performance in one ALS patient correlates with that of their affected relative needs to be determined. This may be extended further to examine whether cognitive performance in relatives correlates with that of patients from their kindred. Finally, whether certain cognitive and neuropsychiatric profiles cluster in the relatives and probands from certain kindreds would be of great interest, as such families may be most informative in genetic studies.

As we enter an era of new genetic therapeutic options for ALS and other neurodegenerative disorders, interest is understandably growing in comprehensively characterising pre-symptomatic or early-stage gene carriers, in the hope that earlier intervention may yield greater therapeutic success. The current Strong criteria for describing cognitive and behavioural changes in ALS provides cross-sectional classification criteria based on severity of symptoms in patients. Yet, work is needed to encompass these cognitive and behavioural criteria into ALS motor staging systems, to allow for monitoring of progression of both cognitive and motor symptoms in respect to defined clinical milestones. This expansion should seek also to encompass the genetically ‘at-risk’ population, who have been shown in this project to exhibit considerable early and subtle cognitive and neuropsychiatric changes. Through integration of this neurocognitive signal with neurophysiological evidence of neural
network disturbance, we can develop biomarker profiles which will enable the recategorization of ALS and associated disorders into precise constellations of aberrant neural pathophysiology. This work may help with identifying factors resulting in apparent reduced penetrance for certain pathogenic mutations. Knowledge of such protective factors will assist in pinpointing interventions that can be instigated in this population to mitigate disease risk.

Finally, a longitudinal study is warranted to follow up on the findings from my cross-sectional analysis of cognitive and neuropsychiatric changes in relatives of those with ALS. In particular, assessment of the temporal stability of putative endophenotypes is required. The cohort of young relatives recruited for this project provides an excellent starting point for such a study. This would be strengthened further by recruitment of greater numbers of C9orf72 repeat expansion carriers, particularly those who are older but otherwise well. That being true, a novel finding from this project was the extent of cognitive changes non-carrier relatives from C9orf72 kindreds, a cohort often used as a default control groups in studies, neglecting the opportunity to study them in their own right. As ALS is an oligogenic disorder, it is highly probably that other ALS associated genes run in these families which may make a substantial impact on the phenotypic expression. To gain greater insight into the distinct and overlapping phenotypic features of both cohorts, I recommend that future studies of asymptomatic gene carriers be compared not only to asymptomatic non-carrier relatives, but also to a healthy control population.

In conclusion, this project has comprehensively studied genetic risk in ALS, and how this influences phenotypic manifestations within kindreds. Several families have been identified with high degrees of shared genetic risk between ALS and other neuropsychiatric disorders. It is hoped that insights gained from this research will ultimately lead to greater understanding of the underlying pathobiological process at play in these families, and help to identify novel therapeutic targets.
14. References


289
39. National Institute for Health and Care Excellence (NICE) Guidance on the use of Riluzole (Rilutek) for the treatment of Motor Neurone Disease


132. Amyotrophic Lateral Sclerosis online Database (ALSOD) [online]. Available at: https://alsod.ac.uk/.


Gordon PH, Delgadoño D, Piquard A, et al. The range and clinical impact of cognitive impairment in French patients with ALS: a cross-sectional study of neuropsychological test


Armon C. Smoking may be considered an established risk factor for sporadic ALS. Neurology 2009;73:1693-1698.


Wingo TS, Cutler DJ, Yarab N, Kelly CM, Glass JD. The heritability of amyotrophic lateral sclerosis in a clinically ascertained United States registry research project. PloS one 2011;6:e27985.


407. Burke T. Delineating the impact of executive dysfunction on social cognition for patients and caregivers in Amyotrophic Lateral Sclerosis Trinity College Dublin, 2016.
419. Meyers JE, Meyers KR. Rey Complex Figure Test and Recognition Trial: Psychological Assessment Resources, Odessa., 1995.
452. Osterrieth PA. Filetest de copie d'une figure complex: Contribution a l'etude de la perception et de la memoire. Archives de Psychologie 1944;30:286-356.


Valdmanis PN, Rouleau GA. Genetics of familial amyotrophic lateral sclerosis. Neurology 2008;70:144-152.


Lule D, Ludolph AC, Ludolph AG. Neurodevelopmental and neurodegenerative diseases - is there a pathophysiological link? Attention-deficit/hyperactivity disorder and amyotrophic lateral sclerosis as examples. Medical hypotheses 2008;70:1133-1138.


Tarter RE. Are there inherited behavioral traits that predispose to substance abuse? Journal of consulting and clinical psychology 1988;56:189-196.


546. ZIEGLER LH. PSYCHOTIC AND EMOTIONAL PHENOMENA ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS. Archives of Neurology & Psychiatry 1930;24:930-936.
551. Byrne RP. Characterising the effects of sex interaction, pleiotropy and local population structure on ALS GWAS [Doctor of Philosophy]: Trinity College Dublin, 2021.


**Appendix 1: Neuropsychological batteries used in the assessment of Irish ALS patients**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intelligence Quotient</strong></td>
<td>Test of Premorbid Function UK (TOPF)</td>
<td>Test of Premorbid Function UK (TOPF)</td>
<td>Raven’s Coloured Progressive Matrices</td>
</tr>
<tr>
<td></td>
<td>Raven’s Coloured Progressive Matrices</td>
<td>Raven’s Coloured Progressive Matrices</td>
<td>Wechsler Test of Adult Reading (WTAR)</td>
</tr>
<tr>
<td><strong>Executive Function</strong></td>
<td>Verbal Fluency</td>
<td>Verbal Fluency</td>
<td>Verbal Fluency</td>
</tr>
<tr>
<td></td>
<td>Digit Span</td>
<td>Digit Span</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colour-Word Interference Test</td>
<td>Colour-Word Interference Test</td>
<td>Colour-Word Interference Test</td>
</tr>
<tr>
<td></td>
<td>Sorting Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brixton Spatial Anticipation Test</td>
<td>Brixton Spatial Anticipation Test</td>
</tr>
<tr>
<td><strong>Social Cognition</strong></td>
<td>Conflicting Emotional Prosody</td>
<td>Conflicting Emotional Prosody</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reading the Mind in the Eyes test</td>
<td>Reading the Mind in the Eyes test</td>
<td></td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td>Boston Naming Test</td>
<td>Boston Naming Test</td>
<td>Boston Naming Test</td>
</tr>
<tr>
<td></td>
<td>Action Naming Test</td>
<td>Sydney Naming Battery</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyramids and Palm Tree Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psycholinguistic Assessments of Language Processing in Aphasia (PALPA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td>Rey Auditory Verbal Learning Test</td>
<td>Rey Auditory Verbal Learning Test</td>
<td>Logical Memory (WMS-IV)</td>
</tr>
<tr>
<td></td>
<td>Logical Memory (WMS-IV)</td>
<td>Logical Memory (WMS-IV)</td>
<td>Verbal Paired Associate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Auditory Delayed Recognition Task</td>
</tr>
<tr>
<td><strong>Visuospatial</strong></td>
<td>Rey Complex Figure Test</td>
<td>Rey Complex Figure Test</td>
<td>Rey Complex Figure Test</td>
</tr>
<tr>
<td><strong>Behaviour</strong></td>
<td>Beaumont Behavioural Inventory</td>
<td>Beaumont Behavioural Inventory</td>
<td>Frontal Systems Behaviour Scale</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Frontal Systems Behaviour Scale</td>
</tr>
<tr>
<td><strong>Mood</strong></td>
<td>Hospital Anxiety and Depression Scale</td>
<td>Hospital Anxiety and Depression Scale</td>
<td>Hospital Anxiety and Depression Scale</td>
</tr>
</tbody>
</table>
Appendix 2: Family histories of C9orf72-positive patients who did not meet Byrne criteria for FALS.

<table>
<thead>
<tr>
<th>No.</th>
<th>Family History Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No information available on family.</td>
</tr>
<tr>
<td>2</td>
<td>No information available on second-degree relatives.</td>
</tr>
<tr>
<td>3</td>
<td>2 first-degree relatives had dementia.</td>
</tr>
<tr>
<td>4</td>
<td>No information available on second-degree relatives.</td>
</tr>
<tr>
<td>5</td>
<td>No information available on second-degree relatives.</td>
</tr>
<tr>
<td>6</td>
<td>1 first-degree relative and 1 second-degree relative committed suicide.</td>
</tr>
<tr>
<td>7</td>
<td>No information available on family.</td>
</tr>
<tr>
<td>8</td>
<td>1 first-degree relative had dementia and 1 third-degree relative had multiple sclerosis.</td>
</tr>
<tr>
<td>9</td>
<td>No information available on family.</td>
</tr>
<tr>
<td>10</td>
<td>1 first-degree relative had Parkinson’s Disease. 1 second-degree relative had Parkinson’s Disease. 1 second-degree relative had dementia.</td>
</tr>
<tr>
<td>11</td>
<td>No information available on paternal side of family. 1 second-degree relative committed suicide.</td>
</tr>
<tr>
<td>12</td>
<td>1 first-degree relative and 1 second-degree relative had schizophrenia.</td>
</tr>
<tr>
<td>13</td>
<td>4 out of 6 siblings died under the age of 40. Incomplete information available on second-degree relatives.</td>
</tr>
<tr>
<td>14</td>
<td>3 second-degree relatives had Alzheimer’s dementia.</td>
</tr>
<tr>
<td>15</td>
<td>1 first-degree relative had dementia.</td>
</tr>
<tr>
<td>16</td>
<td>1 second-degree relative had dementia.</td>
</tr>
<tr>
<td>17</td>
<td>3 first-degree relatives had dementia and 1 second-degree relative had autism.</td>
</tr>
<tr>
<td>18</td>
<td>1 first-degree relative had schizophrenia. 1 second-degree relative had schizophrenia. 2 third-degree relatives committed suicide. 1 second-degree relative had neuropsychiatric disorder (unspecified).</td>
</tr>
<tr>
<td>19</td>
<td>2 first-degree relatives with Bipolar affective disorder, one of whom committed suicide.</td>
</tr>
<tr>
<td>20</td>
<td>3 second-degree relatives with neuropsychiatric disorders (unspecified).</td>
</tr>
<tr>
<td>21</td>
<td>1 first-degree relative had alcohol dependence disorder.</td>
</tr>
<tr>
<td>22</td>
<td>1 first-degree relative and 1 second-degree relative had Alzheimer’s dementia. 2 second-degree relatives had Parkinson’s Disease. 1 second-degree relative and 4 third-degree relatives had alcohol dependence disorder.</td>
</tr>
<tr>
<td>23</td>
<td>4 first-degree relatives had alcohol dependence disorder.</td>
</tr>
<tr>
<td>24</td>
<td>1 first-degree relative had dementia.</td>
</tr>
<tr>
<td>25</td>
<td>2 second-degree relatives had neuropsychiatric disorders (unspecified).</td>
</tr>
<tr>
<td>26</td>
<td>1 second-degree relative had Alzheimer’s dementia.</td>
</tr>
<tr>
<td>27</td>
<td>1 first-degree relative had schizophrenia. 1 first-degree relative had bipolar affective disorder. 1 second-degree relative had alcohol dependence disorder.</td>
</tr>
<tr>
<td>28</td>
<td>1 first-degree relative had dementia. 2 first-degree relatives had alcohol dependence disorder. 1 second-degree relative had neuropsychiatric disorder (unspecified). 1 second-degree relative had multiple sclerosis.</td>
</tr>
<tr>
<td>29</td>
<td>1 first-degree relative and 2 second-degree relatives had Alzheimer’s dementia.</td>
</tr>
<tr>
<td>30</td>
<td>1 first-degree relative had dementia.</td>
</tr>
<tr>
<td>31</td>
<td>1 first-degree relative had schizophrenia and alcohol dependence disorder. Suspect 1 second-degree relative had ALS.</td>
</tr>
<tr>
<td>32</td>
<td>1 first-degree relative and 3 second-degree relatives had dementia. 1 third-degree relative had Parkinson’s Disease. Suspect 1 second-degree relative had ALS.</td>
</tr>
<tr>
<td>33</td>
<td>1 second-degree relative had dementia. Suspect first-degree relative had ALS.</td>
</tr>
<tr>
<td>34</td>
<td>Suspect first-degree relative had ALS.</td>
</tr>
<tr>
<td>35</td>
<td>1 first-degree relative had Bipolar affective disorder. Suspect first-degree relative had ALS-FTD and second-degree relative had ALS.</td>
</tr>
</tbody>
</table>
Appendix 3: Expanded Familial ALS Kindreds

The following definitions were used to define Classical familial ALS, Expanded familial ALS (with schizophrenia), Expanded familial ALS (with FTD) and sporadic ALS for the purposes of this comparative analysis.

Table 1: Definitions of classical and expanded FALS criteria and sporadic ALS

<table>
<thead>
<tr>
<th></th>
<th>Classical FALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband with ALS</td>
<td>AND</td>
</tr>
<tr>
<td></td>
<td>≥ 2 first or second-degree relatives with ALS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Expanded FALS (with schizophrenia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband with ALS</td>
<td>AND</td>
</tr>
<tr>
<td></td>
<td>≥1 first-degree relative with schizophrenia</td>
</tr>
<tr>
<td></td>
<td>or</td>
</tr>
<tr>
<td></td>
<td>≥ 2 first- or second-degree relatives with schizophrenia</td>
</tr>
<tr>
<td></td>
<td>or</td>
</tr>
<tr>
<td></td>
<td>≥ 2 first- or second-degree relatives with either ALS or schizophrenia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Expanded FALS (with FTD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband with ALS</td>
<td>AND</td>
</tr>
<tr>
<td></td>
<td>≥1 first- or second-degree relative with FTD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sporadic ALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband with ALS</td>
<td>AND</td>
</tr>
<tr>
<td></td>
<td>No ALS, FTD or schizophrenia</td>
</tr>
<tr>
<td></td>
<td>No significant neuropsychiatric disordersa</td>
</tr>
<tr>
<td></td>
<td>No ALS-associated geneb</td>
</tr>
</tbody>
</table>

a: as suggested in initial analysis pooled family aggregation study table; b: where DNA samples were available

Composition of familial ALS kindreds

The demographic and clinical characteristics of 65 “Classical FALS” probands, 48 “Expanded FALS (with schizophrenia)” probands, 22 “Expanded FALS (with FTD)” probands and 1814 sporadic ALS patients were compared Table 1. below. The 65 Classical FALS probands came from 32 distinct kindreds with each proband having at least two first- or second-degree relatives with ALS. The 48 “Expanded FALS (with schizophrenia)” probands, included 41 ALS probands with at least one first-degree relative with schizophrenia. The remaining 7 probands had at least two first-or second-degree relatives with ALS or schizophrenia. The 22 Expanded FALS (with FTD)” probands all had at least one first- or second-degree relative with FTD. Both Expanded FALS cohorts included some ALS patients with relatives with ALS who did not meet the criteria for Classical FALS. The composition of both cohorts with respect to numbers of relatives with ALS, FTD and schizophrenia are described in Figure 1.
Figure 1. Composition of Expanded Familial ALS cohorts

Expanded FALS (with schizophrenia)

- One relative SCZ, no relatives ALS: 28
- One relative SCZ, one relative ALS: 5
- One relative SCZ, multiple relatives ALS: 4
- Multiple relatives SCZ, no relatives ALS: 3
- Multiple relatives SCZ, one relative ALS: 8

Expanded FALS (with FTD)

- One relative FTD, no relatives ALS: 14
- One relative FTD, one relative ALS: 4
- Multiple relatives FTD, no relatives ALS: 4
IMPORTANCE Heritability describes the proportion of variance in the risk of developing a condition that is explained by genetic factors. Although amyotrophic lateral sclerosis (ALS) is known to have a complex genetic origin, disease heritability remains unclear.

OBJECTIVES To determine the extent of ALS heritability and assess the association of sex with disease transmission.

DESIGN, SETTING, AND PARTICIPANTS A prospective population-based parent-offspring heritability study was conducted from January 1, 2008, to December 31, 2017 to assess ALS heritability, and was the first study to assess heritability in the context of known gene mutations of large effect. A total of 1123 incident cases of ALS, diagnosed according to the El Escorial criteria and recorded on the Irish ALS register, were identified. Ninety-two individuals were excluded (non-Irish parental origin [n = 86] and familial ALS [n = 6]), and 1117 patients were included in the final analysis.

MAIN OUTCOMES AND MEASURES Annual age-specific and sex-specific standardized ALS incidence and mortality-adjusted lifetime risk were determined. Sex-specific heritability estimates were calculated for the overall study cohort, for those known to carry the C9orf72 (OMIM 614260) variant, and for those with no known genetic risk.

RESULTS A total of 32 parent-child ALS dyads were identified during the study period. Affected offspring were younger at the onset of disease (mean age, 52.0 years; 95% CI, 48.8-55.3 years) compared with their parents (mean age, 69.6 years; 95% CI, 62.4-76.9 years; \( P = .008 \)). Lifetime risk of developing ALS in first-degree relatives of individuals with ALS was increased compared with the general population (1.4% [32 of 2234] vs 0.3% [2.6 of 1000]; \( P < .001 \)). Mean lifetime heritability of ALS for the overall study cohort was 52.3% (95% CI, 42.9%-61.7%) and 36.9% (95% CI, 19.8%-53.9%) for those with no known genetic risk. Heritability estimates were highest in mother-daughter pairings (66.2%; 95% CI, 58.5%-73.9%).

CONCLUSIONS AND RELEVANCE This population-based study confirms that up to 50% of variance in ALS has a genetic basis, and that the presence of the C9orf72 variant is an important determinant of heritability. First-degree relatives of individuals with ALS without a known genetic basis remain at increased risk of developing ALS compared with the general population. A higher heritability estimate in mother-daughter pairings points to a sex-mediated effect that has been previously unrecognized.
Amyotrophic lateral sclerosis (ALS), a multisystem neurodegenerative disease, results from a combination of environmental factors and time that interact with pre-existing genetic characteristics. Evidence in support of a genetic origin of the disease includes familial aggregation of the disorder and the description of 30 or more genetic mutations that cosegregate with the clinical phenotype. Of these, 4 gene variants account for 55% of familial ALS cases in European populations.

Although familial clustering of the disease implies the possibility of common genetic causality, it does not indicate the extent to which inherited genetic factors contribute to the phenotype. An alternative measure is heritability, which reflects the proportion of the variation in the risk of developing disease (liability) that is attributable to genetic factors. Viewing the problem in this manner requires an alternative approach to the more familiar binary model of diagnostics (ie, that individuals either have a disease or they do not). The liability model assumes an underlying normally distributed susceptibility for the disease within a population, where an individual’s total risk reflects the combination of genetic and nongenetic factors acting to alter an individual’s chance of developing a disease. In this model, the disease manifests when the “threshold of liability,” a fixed point on the scale of overall risk, has been exceeded (Table 1).

The classic approach toward assessment of heritability examines the difference in disease concordance between monozygotic and dizygotic twins, with higher concordance in the former implying a strong genetic influence. The challenge with this approach for rare conditions lies in the available numbers of twins. Furthermore, in late-onset diseases such as ALS, the covariance among twins may vary with time, as an unaffected twin may develop the disease at a later stage. By contrast, pedigree studies can achieve greater numbers by assessing differences in phenotype presentation across parent-offspring trios to determine narrow-sense heritability, which indicates the extent to which an exhibited phenotype will segregate with the clinical phenotype. Of these, 4 gene variants account for 55% of familial ALS cases in European populations.

Although familial clustering of the disease implies the possibility of common genetic causality, it does not indicate the extent to which inherited genetic factors contribute to the phenotype. An alternative measure is heritability, which reflects the proportion of the variation in the risk of developing disease (liability) that is attributable to genetic factors. Viewing the problem in this manner requires an alternative approach to the more familiar binary model of diagnostics (ie, that individuals either have a disease or they do not). The liability model assumes an underlying normally distributed susceptibility for the disease within a population, where an individual’s total risk reflects the combination of genetic and nongenetic factors acting to alter an individual’s chance of developing a disease. In this model, the disease manifests when the “threshold of liability,” a fixed point on the scale of overall risk, has been exceeded (Table 1).

The classic approach toward assessment of heritability examines the difference in disease concordance between monozygotic and dizygotic twins, with higher concordance in the former implying a strong genetic influence. The challenge with this approach for rare conditions lies in the available numbers of twins. Furthermore, in late-onset diseases such as ALS, the covariance among twins may vary with time, as an unaffected twin may develop the disease at a later stage. By contrast, pedigree studies can achieve greater numbers by assessing differences in phenotype presentation across parent-offspring trios to determine narrow-sense heritability, which indicates the extent to which an exhibited phenotype will be transmitted from parent to offspring. This method is of greater practical use for predictive purposes such as those required for genetic counseling. Estimates of ALS heritability also provide guidance as to the value of the ongoing search for new ALS-associated genes.

Amyotrophic lateral sclerosis heritability has been estimated using twin, pedigree, and population-level genome-wide single-nucleotide polymorphism (SNP) data. Twin studies using monozygotic and dizygotic twin pairs drawn from overlapping clinic and population-based ALS cohorts provided estimates of heritability of between 38% and 85%. A pedigree study using data ascertained from a clinic-based registry estimated heritability to be 40% and 60%. However, heritability estimates could only be calculated using prevalence data taken from the relevant literature, rather than from an equivalent population-based data set.

Studies using genome-wide SNP data in large case-control cohorts with the largest data sets to assess ALS heritability have yielded estimates in the range of 7.2% to 9.5%. These estimates are limited to additive genetic variance that can be captured by common genetic variants genotyped on an SNP array. These lower heritability estimates from SNP data likely reflect a predominantly rare variant-mediated genetic architecture in ALS, a hypothesis that is supported by enrichment of heritability in lower-frequency common SNPs.

In this study, we have used a pedigree approach to estimate heritability based on parent-offspring concordance with reference to a liability threshold for disease, determined from incident data. More important, the general population sampled to determine incidence must be representative of the population from which individuals with ALS and their parents were drawn. To examine heritability, it is also necessary to include an assessment as to whether the annual incidence of ALS varied significantly with time. This assessment has been possible, as the IRISH ALS Register has been in operation for more than 25 years. The purpose of this study was to assess for evidence of temporal change in annual ALS incidence, to determine the current lifetime risk of developing ALS in a well-characterized population, to estimate the heritability of ALS in the same population, and to determine whether heritability is modulated by sex.

Methods

Data Collection

The Irish ALS register, established in 1994, has assembled demographic and clinical data on all individuals who received a diagnosis of ALS in Ireland since its foundation, as described previously. In brief, individuals with a confirmed diagnosis of definite, probable, possible, or laboratory-supported probable ALS according to the El Escorial criteria complete a structured telephone interview in which demographic and clinical details, including familial status, are gathered. For those unable to complete the interview or those identified posthumously, the data are collected from a nominated family member. Since 2008, detailed family aggregation studies examining associated neurodegenerative and neuropsychiatric disorders among relatives of individuals with ALS have provided additional family history information. All participants provided informed written consent for the study. This study was
Table 1. Description of Key Concepts Discussed With Explanation and Justification of Methodological Approaches Used in This Study

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Formula</th>
<th>Data Required</th>
<th>Alternative Approaches</th>
<th>Justification for Approach Chosen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Incidence rate Rate of new cases per population at risk, during a specified time period</td>
<td>No. of disease onsets divided by sum of person years at risk</td>
<td>(1) No. of ALS cases diagnosed annually, considering 5-y age groups 15 to ≤85 y; (2) size of population at risk (census data); (3) standardized to US 2010 population</td>
<td>NA</td>
<td>Prerequisite for calculating lifetime risk; general population sampled must be representative of population from which individuals with ALS and their parents were drawn; to examine this, it is also necessary to determine whether annual incidence of ALS varied significantly with time</td>
</tr>
<tr>
<td></td>
<td>Birth cohort-specific life expectancy Mean number of years a cohort is expected to live, based on year of birth, age, and sex</td>
<td>Data extracted from population-specific period life expectancy tables for specific birth cohorts</td>
<td>(1) Mean year of birth for patients and their parents; (2) period life expectancy at various ages for population under study</td>
<td>NA</td>
<td>Prerequisite for calculating lifetime risk.</td>
</tr>
<tr>
<td></td>
<td>Lifetime risk Likelihood that a person will develop a certain disease during his or her lifetime</td>
<td>Current probability method</td>
<td>(1) Age-specific and sex-specific ALS incidence rates; (2) age structure of population; (3) birth cohort specific life-expectancies</td>
<td>Cumulative risk •Does not take other competing risks into account •Tendency to overestimate the probability of developing a disease</td>
<td>Current probability method •Criterion standard estimate of lifetime risk •Considers competing mortality risks</td>
</tr>
<tr>
<td></td>
<td>Concordance and covariance Concordant parent-offspring pairs consist of proband with ALS whose parent also has ALS</td>
<td>No. of concordant pairs divided by total No. of pairs, where pairs mean sex-specific parent-offspring pairs</td>
<td>(1) No. of concordant parent-offspring pairs; (2) total No. of parent-offspring pairs</td>
<td>NA</td>
<td>Prerequisite for calculating heritability of a binary trait using Falconer’s method</td>
</tr>
<tr>
<td></td>
<td>Threshold of liability Point on scale of liability above which all individuals are affected and below which all are unaffected</td>
<td>Standard normal deviate exceeded by sex-specific lifetime risk in population under study</td>
<td>(1) Sex-specific lifetime risk</td>
<td>NA</td>
<td>Prerequisite for calculating heritability of a binary trait</td>
</tr>
<tr>
<td></td>
<td>Liability model Liability model assumes underlying normally distributed susceptibility for disease within population, where an individual’s liability reflects the combination of genetic and nongenetic factors acting to alter an individual’s risk of developing disease</td>
<td>Modeled as standard normal distribution with mean 0 and variance 1</td>
<td>NA</td>
<td>Binary model •An individual either has the disease or not based on predefined diagnostic criteria</td>
<td>Liability model allows for an estimation of variance in liability within a population, which is required to estimate heritability using a pedigree approach; requires no prior knowledge about underlying genetic risk factors</td>
</tr>
<tr>
<td>Step 3</td>
<td>Heritability Proportion of variance in liability that is attributable to additive genetic factors</td>
<td>Falconer’s method</td>
<td>(1) No. of affected individuals in sample; (2) total No. of individuals in sample; (3) lifetime risk; (4) sex-specific parent-offspring concordance rates</td>
<td>Twin studies •Covariance among twins may vary with time •Difficult recruiting sufficient numbers of twin in rare diseases SNP data •Limited to additive genetic variance captured by common genetic variants genotyped on a SNP array</td>
<td>Pedigree studies •Increased power •Concordance unlikely to vary •Assessment of variation in liability attributable to genetic factors not limited by current testing methods</td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; NA, not applicable.

a Narrow-sense heritability reflects the proportion of variance in liability attributable to additive genetic variance.
b Sex-specific pairs (daughter-mother, son-mother, daughter-father, and son-father).
approved by the Beaumont Hospital Research Ethics Committee (15/40).

**Inclusion and Exclusion Criteria**

All incident cases of ALS cases recorded in the Irish ALS register between January 1, 1995, and December 31, 2017, were included in the study for the purpose of assessing ALS incidence and lifetime risk. Only those diagnosed between January 1, 2008, and December 31, 2017, were included in deriving estimates of heritability, to optimize quality of family history information gathered and minimize potential biases associated with long-running registers. Furthermore, if more than 1 member of the same family received a diagnosis of ALS during this period, the individual with the most recent diagnosis was included in heritability calculations. Individuals with non-Irish parental origin were excluded.

**Determining the Heritability of ALS**

To estimate the heritability of ALS within a population (step 3), it is necessary first to determine the current lifetime risk of developing ALS within this population (step 2). Prior to determining the current lifetime risk of developing ALS, the annual incidence if ALS within this population must be determined (step 1) (Table 1).

**Step 1: Annual ALS Incidence and Temporal Trends**

Annual age-specific and sex-specific standardized ALS incidence rates were calculated using Irish population data averaged for 5 census years (1996, 2002, 2006, 2011, and 2016) and considering 5-year age groups from 15 to 85 years or older. The US 2010 population was used for standardization purposes, as it is the most frequently used standard population in ALS.

To estimate temporal trends in ALS incidence, a simple linear regression line was fitted using calendar year as the independent variable, assessing both overall and sex-specific incidence rates. Model assumptions were assessed using a normal P-P plot, which showed no deviations, and a residual scatterplot, which demonstrated no systematic variation (standard residual range, −1.4 to 2.2).

**Step 2: Lifetime Risk of ALS**

The current probability method provides the criterion standard estimate of lifetime risk, as it considers competing mortality risks. Using this method, estimations of the number of cases of ALS that would develop in specific birth cohorts were calculated on the basis of the person-years at risk, drawn from the Irish life table from 2010-2012 and the age-specific and sex-specific ALS incidence rates, calculated above.

Potential birth cohorts were identified for patients with ALS and their parents. The mean year of birth for patients was 1946 (men, 1947; and women, 1944) and the mean life expectancy for those surviving past childhood for the patient cohort was 71 years for men and 74 years for women. The mean year of birth for patients’ parents was 1923 (men, 1920; and women, 1925) and the mean life expectancy for those surviving childhood was 66 years for men and 67 years for women. Sex-specific lifetime risk estimates of developing ALS were calculated using these life expectancy estimates and a combined mean calculated.

**Risk of ALS in Relatives of Patients**

All parents of patients with ALS who had also received a diagnosis of ALS were identified. Where possible, affected parents were examined at the Irish national ALS clinic in Beaumont Hospital. For affected parents identified posthumously, diagnosis was confirmed using death certificates. The sex of affected parents was identified and matched with the sex of their affected offspring. Concordance rates were calculated by dividing the number of sex-specific ALS concordant pairs by the total number of sex-specific pairs (daughter-mother, son-mother, daughter-father, and son-father).

**Step 3: Heritability of ALS**

Point estimates of heritability and their SEs were derived using formulas proposed by Falconer. The threshold of liability was set using lifetime risk as calculated, for both patients and their parent’s generations. The parent-offspring regression coefficient is the difference in the sex-specific deviation of the mean liability of both parents and offspring from the threshold (xgp − xgr) as a proportion of the mean deviation of affected individuals from the population mean (αg). Subscripts denote the sex-specific lifetime risk in the general population (g) and in relatives (r). Narrow-sense heritability is equal to twice the estimate of the parent-offspring regression coefficient. Calculations were performed for each sex-specific parent-offspring pairing independently, with mean heritability and SE estimates calculated weighted by the reciprocal of the sampling variance for each sex-specific parent-offspring pairing.

**Genetic Screening**

The Irish ALS DNA biobank has been in operation since 1999. To date, only the C9orf72 (OMIM 614260) hexanucleotide repeat expansion has been observed to be a significant genetically identifiable cause of ALS in the Irish population, accounting for 33% of all known familial cases of ALS. C9orf72 repeat expansion testing is performed using repeat-primed polymerase chain reaction plus amplicon length analysis as previously described, with expansions of 30 or more repeats deemed positive. Sex-specific ALS concordance rates were calculated as above including only those in whom C9orf72 status was established and used to determine heritability estimates for the C9orf72-negative population. Five previously identified patients with sporadic ALS who carried other putative ALS-causative gene mutations (TARDBP [OMIM 605078; n = 1], FUS [OMIM 137070; n = 2], SODI [OMIM 147450; n = 1], and SQSTM1 [OMIM 601530; n = 1]) were excluded from analysis.

**Statistical Analysis**

The frequency and percentage were calculated for normally distributed nominal variables and the mean value and SD were calculated for normally distributed continuous variables. A χ² test was used to determine the degree of association between 2 nominal variables. The probability of difference between 2 variables was tested using independent-samples t tests and
probability of difference between 3 variables was tested using one-way analysis of variance with Bonferroni correction. \( P < .05 \) was considered statistically significant. All analyses were conducted using IBM SPSS Statistics, version 24 (IBM Corp).

### Results

#### Clinical Characteristics
Comparison of demographics for both the overall incidence cohort (1995-2017) and the heritability subcohort (2008-2017) are presented in **Table 2**. The higher incidence of cognitive-onset disease observed in the heritability subcohort likely reflects recent improved recognition of this phenomenon. Of those with a parental history of ALS, 17 of 32 (53.1%) were female and 7 of 32 (21.9%) received a diagnosis of cognitive-onset ALS. Individuals with a parental history of ALS had a younger mean age at onset (57.9 years; 95% CI, 55.3-60.6 years) than the remaining heritability subcohort (65.1 years; 95% CI, 64.4-65.9 years; \( P < .001 \)). For 9 cases, in which age at onset was available for both the affected parent and offspring, the affected offspring were younger at time of onset (mean age, 52.0 years; 95% CI, 48.8-55.3 years) compared with their parents (mean age, 69.6 years; 95% CI, 62.4-76.9 years; \( P = .008 \)), despite no difference in time from onset to diagnosis between the groups (10.1 vs 13.7 months; \( P = .41 \)).

#### Incidence and Lifetime Risk of ALS
A total of 2128 patients received a diagnosis of ALS between 1995 and 2017. The annual age-standardized and sex-standardized incidence of ALS did not change with time (0.019; 95% CI, 0.004 to 0.042 per 100 000 persons; \( P = .14 \)). The overall mean incidence rate was 3.1 (95% CI, 2.9-3.2) per 100 000 persons. The mean male-specific annual incidence rate was 1.8 (95% CI, 1.7-1.9) per 100 000 persons and the mean female-specific annual incidence rate was 1.3 (95% CI, 1.2-1.4) per 100 000 persons.

The current lifetime risk of developing ALS, adjusted for other-cause mortality, was 2.9 per 1000 men and 2.3 per 1000 women, corresponding to 1 in 347 men and 1 in 436 women. The mortality-adjusted lifetime risk of developing ALS during the patient’s mean life expectancy was 1.8 per 1000 men and 1.5 per 1000 women. The mortality-adjusted lifetime risk of developing ALS during the patients’ parent’s mean life expectancy was 1.2 per 1000 men and 0.9 per 1000 women. The combined mortality-adjusted lifetime risk for both patients and their parents’ lifespan was 1.5 per 1000 men and 1.2 per 1000 women.

#### Risk in Relatives
Between 2008 and 2017, 1123 incident cases of ALS were identified. A total of 92 individuals were excluded (non-Irish parental origin [n = 86] and familial ALS [n = 6]); 1117 patients were included in final analysis. Complete family history was available for both parents of all patients (n = 2234). A total of 32 parents had a diagnosis of ALS. Total and sex-specific concordance rates are reported in **Table 3**. Concordance was highest in female-female parent-offspring pairs (13 of 491 [2.6%]). Concordance was similar for female-male parent-offspring pairs (4 of 491 [0.8%]) and male-female parent-offspring pairs (5 of 626 [0.8%]). Overall, we observed that in a population devoid of known mendelian-inherited genes, the total lifetime risk of developing ALS was 0.7% (9 of 1210) in any first-degree relative of an individual with ALS. The lifetime risk of developing ALS in first-degree relatives of individuals with ALS whose genetic status is unknown is 1.4% (32 of 2234) (Table 3).

#### C9orf72
C9orf72 status was available for 674 patients diagnosed between 2008 and 2017. A total of 69 patients were C9orf72 positive; 14 of all C9orf72-positive patients (20.3%) reported a parental history of ALS.

A total of 32 patients had a parental history of ALS; 23 of these patients had C9orf72 testing performed, of whom 14 (60.9%) were C9orf72 positive. No other definitive ALS-
causing mutation has been identified in these patients.\textsuperscript{19,22} Four of 9 patients (44.4\%) whose fathers had ALS carried the repeat expansion. By contrast, 10 of 14 patients (71.4\%) whose mothers had ALS carried the \textit{C9orf72} repeat expansion.

### Heritability

The overall mean lifetime heritability of ALS for the study cohort was 52.3\% (95\% CI, 42.9\%-61.7\%). Lifetime heritability estimates by sex pairings and genetic status are displayed in the \textbf{Figure} and \textbf{Table 4}. Unlike-sexed relative pairings, that are sex equivalent, provided the most similar heritability estimates (mother-son, 38.2\%; 95\% CI, 26.5\%-49.9\% and father-daughter 34.2\%; 95\% CI, 21.4\%-47.2\%). If ALS is considered as a polygenic disease, heritability should be equal for all parent-offspring pairings. As such, weighing the unlike-sexed relatives’ heritability estimates by the reciprocal of their variance provides us a mean heritability estimate of 36.4\% (95\% CI, 24.2\%-48.9\%). By contrast, heritability was significantly higher in sex-specific pairings (combined mother-daughter and father-son pairings, 59.3\%; 95\% CI, 51.2\%-67.5\%; $P = .002$). Overall heritability estimates were highest in mother-daughter pairings (66.2\%; 95\% CI, 58.5\%-73.9\%).

In \textit{C9orf72}-negative patients, the overall mean lifetime heritability of ALS was 36.9\% (95\% CI, 19.8\%-53.9\%) (Table 4). Female-specific heritability was 48.5\% (95\% CI, 33.2\%-63.8\%). Heritability estimates between mother-son pairings were nonsignificant, which may be partially attributable to the low number of concordant ALS pairs in this group.

### Discussion

We have conducted the largest population-based pedigree study to date, to our knowledge, to determine ALS heritability. We observed a mean annual age-standardized and sex-standardized ALS incidence and current lifetime risk of developing ALS, similar to those observed by other methods. Our population-based heritability estimates of between 43\% and 62\% are similar to those calculated by Wingo et al\textsuperscript{4} (40\%-60\%) using a US clinic-based population. However, in our study, ALS heritability was assessed for the first time in a population in whom known genetic mutations have been excluded. We found that heritability estimates were not significantly different in \textit{C9orf72}-negative patients when compared with the overall heritability study cohort. Although the lifetime risk of developing ALS in first-degree relatives of individuals with ALS whose genetic status is unknown is 1.4\% (32 of 2234), first-

### Table 3. Parent-Offspring Amyotrophic Lateral Sclerosis Concordance Rates by Overall Heritability Cohort and \textit{C9orf72}-Negative Subcohort

<table>
<thead>
<tr>
<th>Proband Parent</th>
<th>Total Concordance, No./Total No. (%)</th>
<th>\textbf{Heritability Cohort (n = 1117)}</th>
<th>\textbf{C9orf72-Negative Cohort (n = 605)}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Female</td>
<td>13/491 (2.6)</td>
<td>3/232 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Male Female</td>
<td>5/626 (0.8)</td>
<td>1/373 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Female Male</td>
<td>4/491 (0.8)</td>
<td>2/232 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Male Male</td>
<td>10/626 (1.6)</td>
<td>3/373 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32/2234 (1.4)</td>
<td>9/1210 (0.7)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Exact Heritability Estimates per Study Cohort and Offspring-Parent Relationship

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Heritability, % (95% CI)</th>
<th>Overall Population</th>
<th>\textit{C9orf72}-Negative Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>52.3 (42.9 to 61.7)</td>
<td>36.9 (19.8 to 53.9)</td>
<td></td>
</tr>
<tr>
<td>Daughter-mother</td>
<td>66.2 (58.5 to 73.9)</td>
<td>48.5 (33.2 to 63.8)</td>
<td></td>
</tr>
<tr>
<td>Son-mother</td>
<td>38.2 (26.5 to 49.9)</td>
<td>15.2 (-9.5 to 40.0)</td>
<td></td>
</tr>
<tr>
<td>Daughter-father</td>
<td>34.3 (21.4 to 47.2)</td>
<td>35.5 (17.3 to 53.8)</td>
<td></td>
</tr>
<tr>
<td>Son-father</td>
<td>50.1 (42.0 to 59.4)</td>
<td>34.5 (19.3 to 49.6)</td>
<td></td>
</tr>
</tbody>
</table>

A, Mean heritability estimates for overall patient cohort, with lifetime risk of developing amyotrophic lateral sclerosis of 1.5 per 1000 males and 1.2 per 1000 females. B, Mean heritability estimates for \textit{C9orf72}-negative patient subcohort, with lifetime risk of developing amyotrophic lateral sclerosis of 1.5 per 1000 males and 1.2 per 1000 females. Vertical lines indicate 95\% CIs.
degree relatives of individuals with ALS who are not known to carry any ALS-associated genetic mutations, remain at increased risk of developing ALS (lifetime risk, 0.7%) compared with the general population (lifetime risk, 0.3%).

A total of 14 of 23 (60.9%) concordant ALS pairs in whom DNA was available carried the C9orf72 repeat expansion. By contrast, only 20.3% of C9orf72-positive patients reported a parental history of ALS, suggesting that genetic anticipation or pleiotropic effects may have masked the clinical phenotype in their parents. Moreover, the affected offspring of affected parents were, on average, 17 years younger at age of onset of ALS than their parents.

Across all estimates calculated, mother-daughter pairings had the highest heritability, strongly suggesting a sex-mediated liability in this cohort. Although female sex hormones may modulate risk, it is not possible to determine how these factors may be associated with sex-specific heritability differences. A previous study has shown that both clinical phenotype and survival probability of C9orf72 expansion carriers are also influenced by sex, suggesting a complex interaction between sex, disease phenotype, and the repeat expansion.

Parental sex has been observed to play a role in the stability of other diseases associated with nucleotide expansions. For example, in Huntington disease, an increase in the CAG trinucleotide expansion length occurs almost exclusively through paternal transmission. By contrast, in myotonic dystrophy 1, CTG trinucleotide expansions are more likely to be transmitted maternally. Our observation of higher ALS heritability in females may be in part be driven by C9orf72 repeat expansion, as 71.4% of patients whose mothers had ALS carried the repeat expansion. That female-specific heritability remains elevated in C9orf72-negative populations, suggesting the possibility of additional uncharacterized repeat expansions associated with ALS.

Limitations
This study has several limitations. Although we found no temporal change in the incidence of ALS in Ireland during the most recent 23 years, it was not possible to determine whether incidence has changed during the entire period that patients and their parents were alive. Nonetheless, as ALS is a late-onset disease, the period assessed was that of most interest for these cohorts. Moreover, while the current probable lifetime risk describes the current risk of developing ALS for individuals of a certain age, it does not directly reflect the risk of either patients or parents at the time of their birth, nor is it associated with future risk.

Second, the association of environmental variables with risk of ALS was not directly assessed during this study. Heritability estimates are specific to the population and environment in which they are estimated. Of proposed environmental risk factors associated with ALS, other than smoking, no clear reported change in these variables has occurred over time. Although it is possible that a changing environmental background during the last century may have been associated with heritability estimates, it seems unlikely that this would be the case given the stability of the incidence of ALS within the population.

Finally, while we have accounted for all known genes of large effect in the Irish population, it is not possible to control for unknown genes of large effect. Evidence from genome-wide association studies would suggest that a large proportion of genetic risk is driven by rare variants. Whether these are mutations within the population or isolated occurrences arising de novo in large kindreds is as yet unknown. Nonetheless, the heritability estimates generated by our study support the ongoing search for such mutations to help us explain further the pathogenic mechanisms driving the disease.

Conclusions
To our knowledge, this is the largest population-based pedigree study assessing ALS heritability to date and the first study to assess heritability in the absence of known gene mutations of large effect. We have shown that the overall heritability of ALS in this population is 52.3%. We have found that, in the absence of known ALS-associated genetic mutations in the proband, first-degree relatives of individuals with ALS remain at increased risk of developing ALS compared with the general population. Finally, we consistently observed that the highest heritability estimates occur in mother-daughter pairings, pointing to a previously unrecognized sex-mediated association with disease heritability.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES


Comparison of the clinical and genetic features of amyotrophic lateral sclerosis across Cuban, Uruguayan and Irish clinic-based populations

Marie Ryan, Tatiana Zaldivar Vaillant, Russell L McLaughlin, Mark A Doherty, James Rooney, Mark Heverin, Joel Gutierrez, Gloria Esther Lara-Fernández, Mariana Pita Rodríguez, Jochen Hackembruch, Abayúba Perna, María Cristina Vazquez, Marco Musio, Carlos N Ketzoian, Giancarlo Logroscino, Orla Hardiman

ABSTRACT

Objectives This study compares the clinical characteristics of patients with amyotrophic lateral sclerosis (ALS) within three clinic-based populations from Cuba, Uruguay and Ireland and determines the impact of known ALS-associated genetic variants on phenotypic manifestations within the Cuban population.

Methods Demographic and clinical information was collected on 115 Cuban, 220 Uruguayan and 1038 Irish patients with ALS attending national specialist clinics through 1996–2017. All Cuban patients and 676 Irish patients underwent next-generation DNA sequencing and were screened for the pathogenic C9orf72 repeat expansion.

Results The mean age of onset was younger in the Cuban population (53.0 years, 95% CI 50.4 to 55.6) and Uruguayan population (58.2 years, 95% CI 56.5 to 60.0) populations compared with the Irish population (61.6 years, 95% CI 60.9 to 62.4). No differences in survival between populations were observed. 1.7% (95% CI 0.6 to 4.1) of Cubans with ALS carried the C9orf72 repeat expansion compared with 9.9% (95% CI 7.8 to 12.0) of Irish patients with ALS (p=0.004). Other known variants identified in the Cuban population included ANG (one patient), CHCHD10 (one patient) and DCTN1 (three patients).

Conclusions and relevance This study is the first to describe the clinical characteristics of ALS in Cuban and Uruguayan populations and report differences between the Cuban and Irish genetic signature in terms of known ALS-associated genetic variants. These novel clinical and genetic data add to our understanding of ALS across different and understudied populations.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition of complex genetic and environmental origin characterised by motor system degeneration with variable degrees of extra-motor involvement. To date, the vast majority of epidemiological studies of this condition have been performed in populations of European ancestry.①

In European populations, familial ALS has been reported to occur in 5%–20% of ALS probands.②③④⑤ Of 30+ Mendelian inherited genes associated with ALS,⑥ the most important of these include the C9orf72 repeat expansion, accounting for 6%–10% of all cases,⑤ and variants in SOD1, TDP43 and FUS,⑥ although the relative frequencies of these differ across European populations. By contrast, variants in OPTN are common in Asia but rare in European cohorts.⑦⑧ Furthermore, studies in Asian-Pacific countries, where SOD1 and FUS mutations are relatively common and the C9orf72 repeat expansion is rare,⑦⑧ have reported longer survival periods than in most Western populations,⑨ not accounted for by differences in clinical care alone.⑩

The likelihood that population-specific genetics signatures drive population-specific disease patterns is supported in part by the wide geographical variance in the incidence and prevalence of ALS.①⑩ Evidence of increased genetic susceptibility has been noted in isolated populations (eg, Faroe Islands, Kii Peninsula in Japan and Guam).⑩⑪⑫ Conversely, and notwithstanding the relative lack of epidemiological studies in truly admixed populations, there is evolving evidence to suggest that population admixture may be protective.

Two large population-based mortality studies in truly admixed populations have suggested differential risk within populations of different ancestral origin.⑩⑪⑫ A large Cuban study showed lower ALS rates in those of mixed ancestral origin compared with those of Spanish origin.⑩ Subsequently, a large Chilean population-based mortality study found ALS rates comparable with the Cuban population, with higher mortality rates in geographical populations of predominantly European ancestry compared with the national average.⑩⑫ However, whether other demographic and phenotypic characteristics differ between admixed populations and those of Northern European extraction is not yet known.

Here, we report on the demographic and clinical characteristics of patients with ALS within two large clinic-based populations from the provinces of Havana and its environs in Cuba, and in Montevideo in Uruguay. We compare these with Irish clinic-based population data collected at the same time. We also compare for the first time the frequency of known susceptibility genes in
clinic-based populations from Havana and within the Irish ALS population.

METHODS

Study populations

The National Institute of Neurology, Havana, Cuba is a publicly funded, university-affiliated hospital and tertiary referral system for all of Cuba for neurodegenerative conditions. This centre, alongside other tertiary referral centres, is supported by the secondary health system which encompasses neurological, rehabilitation and palliative services from the 52 provincial and municipal university-affiliated hospitals. Extrapolating from data obtained from a Cuban population-based mortality study and Cuban census data, we estimate that the 115 patients with ALS captured by this clinic likely reflect 20%–30% of all Havana-based patients with ALS diagnosed during our study period. While extrapolating incidence data from mortality studies is not usually reliable, one review comparing incidence versus mortality data in ALS identified this study as one of three high-quality, usually reliable, one review comparing incidence versus mortality data. While extrapolating incidence data from mortality studies is not usually reliable, one review comparing incidence versus mortality data in ALS identified this study as one of three high-quality studies assessing ALS mortality rates, which likely provides an accurate reflection of the population ALS incidence rate.

By contrast, more than half of the Uruguayan population live in its capital city, Montevideo. Here, the Institute of Neurology, Hospital Clinics serves as a national tertiary referral centre for neurodegenerative conditions covering 19 administrative regions. Extrapolating from data obtained from a Uruguayan population-based incidence study, we estimate that the 220 patients with ALS captured by this clinic likely reflect approximately 40% of all Uruguayans diagnosed with ALS and living in Montevideo during our study period. Finally, the Irish national ALS clinic in Beaumont Hospital, Dublin is a public clinic serving the population of Ireland. The majority of individuals diagnosed with ALS in Ireland attend this clinic. The overall population estimates for Cuba, Uruguay and Ireland at the midpoint of this study were 11.2, 3.3 and 4.2 million, respectively.

Data collection

All patients who attended the specialist clinics and were diagnosed with definite, probable or possible ALS as defined by the El Escorial criteria, between 1996 and 2017, were recruited for the study. Those with Progressive Muscular Atrophy (PMA) and Primary Lateral Sclerosis (PLS) were excluded. All patients had detailed histories and complete general and neurological examinations carried out by the attending neurologists. Demographic information collected included date of birth, date of onset, date of diagnosis, date to last follow-up or date of death, self-reported sex, and province of residency. Clinical information collected included site of onset, first clinical symptom and prescription of riluzole. Extensive family pedigrees were detailed from all patients reporting a definite or probable family history of ALS as defined by the Byrne criteria. In line with the official Cuban guidance, Cuban patients in the study were stratified by ethnicity according to self-reported skin colour (‘white’, ‘black’ or ‘mulatto’).

DNA collection

DNA extracted from venous leucocytes were collected from all participating Cuban patients and controls who were phenotypically normal at the time of sampling. Cuban controls included spouses of patients and volunteers recruited nationwide through primary care offices. DNA samples were collected from all patients on the Irish ALS register who attended the Irish national ALS clinic in Beaumont Hospital. Genomic analysis was performed in house in the Smurfit Institute of Genetics, Trinity College Dublin.

Genomic analysis

For 126 Cuban cases, 111 Cuban controls, 404 Irish cases and 310 Irish controls, targeted 300 base pair single-end next-generation DNA sequencing was carried out on an Illumina MiSeq. The exons of 37 genes reliably linked to ALS or dementia (SOD1, ANG, C21orf2, CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERPB4, FIG4, FUS, GRN, hnRNPA1, LMNB1, MAPT, MATR3, NEFH, NEK1, OPTN, PFN1, PRPH, PSEN1, PSEN2, SARM1, SETX, SIGMAR1, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TBK1, UBQLN2, UNCI13A, VAPB, VCP) were targeted. Additionally, 272 Irish patients and 136 Irish controls underwent Illumina PCR free whole genome sequencing. Known ALS variants were defined as those which had an alternate allele frequency below 1% in controls or 5% in reference population data sets and which were present in the ALS Online Genetics Database. Screening for the presence of the pathogenic C9orf72 repeat expansion in all Cuban patients and 832 Irish patients was performed using repeat-primed PCR with amplified fragments measurement by capillary electrophoresis on an Applied Biosystems 3500 Series Genetic Analyzer and visualised using GeneMapper V.4.0, as described previously. A cut-off value of 30 hexanucleotide repeats or above was used to categorise samples as positive for the repeat expansion.

Statistical analysis

Data analysis was carried out for the following variables: age at onset, age at diagnosis, self-reported sex, self-reported ethnicity, family history, site of onset, riluzole prescription, onset to diagnosis interval, survival from onset and survival from diagnosis. The frequency or percentage of nominal variables was calculated. Association between two or more nominal variables was determined using the $\chi^2$ test. Normally and non-normally distributed continuous variables were described with means (SD) and medians (IQR), respectively. The probability of difference between two normally distributed groups was tested using independent-sample t-test and non-normally distributed groups using the Mann-Whitney U test. The probability of a difference between normally distributed variables (age of onset and age of diagnosis) among greater than two groups was tested with the one-way analysis of variance (ANOVA), with Bonferroni post-hoc testing performed to correct for multiple pairwise comparisons. The probability of a difference between non-normally distributed variables (onset to diagnosis interval) among greater than two groups was tested with Kruskal-Wallis rank-order one-way ANOVA. Patients were followed from their time of diagnosis until death or censor date (31 December 2017). For survival analysis, Kaplan-Meier curves were constructed with equality of outcome assessed using the log-rank test. Survival analysis was performed for both censored and uncensored (only those with date of death available) data. Statistical significance was declared for $p<0.05$. Statistical analysis was conducted using IBM SPSS Statistics V24.
Impact of ethnicity on demographic and clinical characteristics of disease

The demographic and clinical characteristics of our study populations are described in table 1.

Allowing for differences in respect to cohort size, considerable variance in respect to age of onset and diagnosis was observed between populations (p<0.001). The Cuban population demonstrated the earliest ages of onset and diagnosis of 53.0 (95% CI 50.4 to 55.6) and 54.4 (95% CI 51.9 to 56.9) years, respectively, with the mean age of onset and diagnosis occurring approximately 9 years later in the Irish population (age of onset 61.6 years [95% CI 60.9 to 62.4]; age of diagnosis 63.3 years [95% CI 62.3 to 63.7]). The age of onset and diagnosis in the Uruguayan population was 58.2 (95% CI 56.5 to 60.0) and 59.5 (95% CI 57.8 to 60.3) years, respectively. Despite lower riluzole prescription rates in the Cuban and Uruguayan populations (riluzole prescription: Cuba 37.5%, Ireland 83.1%, Uruguay 25.1%; p<0.001), no differences in survival durations were observed between populations (survival from diagnosis, months, median [IQR]: Cuba 26.0 (13.0–48.0), Ireland 21.0 (12.0–41.0), Uruguay 23.5 (12.8–42.9); p=0.72). The mean length of follow-up was for Cuba 30 months (95% CI 24.3 to 35.6), for Ireland 27.1 months (95% CI 25.2 to 29.0) and for Uruguay 29.1 months (95% CI 24.7 to 33.5). No differences in sex composition or site of disease onset between populations were observed.

### Impact of ethnicity on demographic and clinical characteristics of disease

The demographic and clinical characteristics of the Cuban population stratified by ethnicity are reported in table 2. The majority of Cuban patients with ALS were of self-reported ‘white’ ethnicity (64.9%). No significant differences, as regards all assessed variables, were observed between groups.

### Genetic signatures (Cuban and Irish comparison)

Of Cuban patients with ALS, 5.2% (5/115) were found to carry previously described disease-associated variants (table 3; ANG 1, CHCHD10 1, DCTN1 3). The pathogenic hexanucleotide repeat expansion in C9orf72 was identified by repeat-primed PCR in two patients, representing 1.7% (95% CI 0.6 to 4.1) of all Cuban patients with ALS (table 3). Both carriers of the C9orf72 repeat expansion and one ANG mutation were detected in the ‘white’ Cuban population. A DCTN1 (c.3746C>T(p. Thr1249Ile)) variant was observed in the ‘black’ Cuban population, while a CHCHD10 (c.100C>T(p.Pro34Ser)) mutation was observed in the ‘mulatto’ population. Two of 96 (2.1%) individuals with sporadic ALS and 0 of 18 patients with familial ALS carried the C9orf72 repeat expansion. The proportion of Cuban patients with ALS was lower than observed in the Irish ALS population, where 82 of 832 (9.9%, 95% CI 7.8 to 12.0) patients were found to carry the repeat expansion (p=0.004). Five additional Irish patients were found to carry known ALS-associated variants (TARDBP 1, FUS 2, SOD1 1, SQSTM1 1). No known SOD1, TARDBP or FUS mutations were found in the Cuban ALS population.

**DISCUSSION**

This study is the first to describe the clinical and demographic characteristics of ALS phenotypes in Cuban and Uruguayan populations, with clinical implications for these groups and for their diaspora. Our findings demonstrate a significantly lower age of onset and diagnosis in these two Latin-American clinic-based cohorts compared with their Irish counterparts. It is generally considered that earlier onset of disease may reflect a major exposure to a risk factor, either genetic or environmental. This hypothesis aligns well with the multistep model of ALS, where recent work has shown a reduced number of steps in patients with ALS with genetic mutations. Age of onset has been shown to vary between populations of different ancestries, with a lower age of onset of ALS observed in some South American, Asian and African populations, which may be partially attributable to lower median ages across these populations (eg, India and most African populations), compared with their European counterparts. However, examination of sex-specific life expectancies at birth and at 50 years of age published in the United Nations demographic yearbook at the midpoint of the study period reveals no notable differences between Cuban, Uruguayan and Irish populations (life expectancies at birth in years: [male] Cuba 75.1, Uruguay 72, Ireland 75.1; [female] Cuba 79, Uruguay 79.5, Ireland 80.3; life expectancies at 50 years: [male] Cuba 28.5, Uruguay 26, Ireland 27.8; [female] Cuba 31.3, Uruguay 32.3, Ireland 31.9).

---

**Table 1** Comparison of demographic and clinical characteristics by population origin

<table>
<thead>
<tr>
<th></th>
<th>Cuba (n=115)</th>
<th>Ireland (n=1038)</th>
<th>Uruguay (n=220)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Continuous variables, mean (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of onset (years)</td>
<td>53.0 (50.4 to 55.6)</td>
<td>61.6 (60.9 to 62.4)</td>
<td>58.2 (56.5 to 60.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age of diagnosis (years)</td>
<td>54.4 (51.9 to 56.9)</td>
<td>62.0 (62.3 to 63.7)</td>
<td>59.5 (57.8 to 60.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Survival from diagnosis (months), censored</td>
<td>39.0 (24.0–70.0)</td>
<td>35.0 (23.0–63.0)</td>
<td>36.9 (22.9–71.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Survival from diagnosis (months), uncensored (date of death available)</td>
<td>30.0 (20.0–55.0)</td>
<td>30.0 (20.0–47.0)</td>
<td>32.0 (21.0–47.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Onset–diagnosis interval (months)</td>
<td>12.0 (7.0–17.0)</td>
<td>11.0 (6.0–19.0)</td>
<td>10.0 (5.1–18.8)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Continuous variables, median (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset–diagnosis interval (months)</td>
<td>12.0 (7.0–17.0)</td>
<td>11.0 (6.0–19.0)</td>
<td>10.0 (5.1–18.8)</td>
<td>0.79</td>
</tr>
<tr>
<td>Survival from onset (months), censored</td>
<td>39.0 (24.0–70.0)</td>
<td>35.0 (23.0–63.0)</td>
<td>36.9 (22.9–71.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Survival from onset (months), uncensored (date of death available)</td>
<td>30.0 (20.0–55.0)</td>
<td>30.0 (20.0–47.0)</td>
<td>32.0 (21.0–47.0)</td>
<td>0.25</td>
</tr>
<tr>
<td>Survival from diagnosis (months), censored</td>
<td>26.0 (13.0–48.0)</td>
<td>21.0 (12.0–41.0)</td>
<td>23.5 (12.8–42.9)</td>
<td>0.72</td>
</tr>
<tr>
<td>Survival from diagnosis (months), uncensored (date of death available)</td>
<td>21.0 (10.0–36.0)</td>
<td>17.0 (10.0–29.0)</td>
<td>19.9 (10.8–34.9)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Nominal variables, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>63 (54.8)</td>
<td>611 (58.9)</td>
<td>136 (61.8)</td>
<td>0.46</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>70 (60.9)</td>
<td>657 (63.3)</td>
<td>146 (66.4)</td>
<td>0.60</td>
</tr>
<tr>
<td>Familial ALS present (yes)</td>
<td>18 (15.8)</td>
<td>122 (11.8)</td>
<td>11 (5.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Riluzole (prescribed)</td>
<td>42 (37.5)</td>
<td>863 (83.1)</td>
<td>55 (25.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Irish clinic-based patients with ALS were available for comparison. Genetic data were available for 832 of these. The demographic and clinical characteristics of our study populations are described in table 1.

C9orf72 repeat expansion and one ANG mutation were detected in the ‘white’ Cuban population. A DCTN1 (c.3746C>T(p. Thr1249Ile)) variant was observed in the ‘black’ Cuban population, while a CHCHD10 (c.100C>T(p.Pro34Ser)) mutation was observed in the ‘mulatto’ population. Two of 96 (2.1%) individuals with sporadic ALS and 0 of 18 patients with familial ALS carried the C9orf72 repeat expansion. The proportion of Cuban patients with ALS was lower than observed in the Irish ALS population, where 82 of 832 (9.9%, 95% CI 7.8 to 12.0) patients were found to carry the repeat expansion (p=0.004). Five additional Irish patients were found to carry known ALS-associated variants (TARDBP 1, FUS 2, SOD1 1, SQSTM1 1). No known SOD1, TARDBP or FUS mutations were found in the Cuban ALS population.
Nonetheless, no significant difference in survival between the Cuban, Uruguayan and Irish cohorts was observed. For comparison purposes, Table 4 details the reported age of onset/diagnosis and survival for other Latin-American/Hispanic populations.29 33–35 Younger age of onset is considered a positive prognostic indicator as is suggested in this table. Indeed, evidence provided by a meta-analysis of genome-wide association samples, encompassing data from 13 European ancestry cohorts, has identified six genomic regions associated with age at onset of ALS.36 This suggests that population-specific genetic factors may influence demographic features across different countries.

Current evidence points to ALS as a multifactorial condition, resulting from a combination of genetic and environmental factors.37 The populations assessed in this study are very different in terms of environmental exposures, including care received following diagnosis. In Cuba and Uruguay, patients

---

**Figure 1** (A) Kaplan-Meier plots of survival probabilities stratified by nationality for (1) onset to death and (2) diagnosis to death. (B) Kaplan-Meier plots of survival probabilities for Cuban amyotrophic lateral sclerosis population stratified by ethnicity for (1) onset to death and (2) diagnosis to death. Cum, cumulative. Log-rank for equality of survival functions: A1) survival from onset stratified by nationality p=0.808. A2) survival from diagnosis stratified by nationality p=0.718. B1) survival from onset stratified by ethnicity p=0.790. B2) survival from diagnosis stratified by ethnicity p=0.625.
attend specialist clinics in Havana and Montevideo, respectively. While both clinics have access to riluzole, non-invasive ventilation (NIV) is not routinely available in Cuba, and is used as part of standard care in Ireland and Uruguay. Furthermore, riluzole prescription rates varied significantly across sites. It is possible that this may have impacted on outcome; however, it should also be noted that no difference in survival from onset was observed across the three populations. The data available did not permit a detailed exploration of risk in the context of environmental exposures and population genetics, and additional comparative studies are required.

However, our study is the first to describe the genetic signature of ALS in a Caribbean population as explained by known ALS-associated variants. Of the Cuban clinic-based population 5.2% had a known ALS-associated genetic variant (ANG 1, CHCHD10 1, DCTN1 3, C9orf72 2). The proportion of Cubans with ALS carrying the C9orf72 repeat expansion was significantly lower than their Irish counterparts (1.7% vs 9.9%, p = 0.004). Indeed, a lesser proportion of Cubans with sporadic ALS carried the C9orf72 repeat expansion (2.1%) compared with the carrier rate reported in a pooled analysis of European sporadic ALS cases (5.1%). In keeping with the known variations in the frequency of C9orf72 repeat expansions across different populations, the proportion of Cubans with sporadic ALS carrying the C9orf72 repeat expansion was comparable with the rate seen in other Latin-American populations (Brazil 3.6%; Argentina 2% and greater than that seen in a pooled analysis of Asian sporadic ALS cases (0.3%)). No known SOD1, TARDBP or FUS mutations were found in the Cuban ALS cohort studied. In contrast, mutations in SOD1, TARDBP and FUS are among the most common genetically identifiable causes for ALS in European and Asian populations.

Table 2 Comparison of variables by self-reported ethnicity (Cuba)

<table>
<thead>
<tr>
<th>Continuous variables, mean (95% CI)</th>
<th>White (n=74)</th>
<th>Black (n=14)</th>
<th>Mulatto (n=26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset (years)</td>
<td>54 (51 to 57)</td>
<td>54 (46 to 63)</td>
<td>50 (44 to 56)</td>
<td>0.48</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>55 (52 to 58)</td>
<td>55 (48 to 63)</td>
<td>51 (46 to 57)</td>
<td>0.45</td>
</tr>
<tr>
<td>Continuous variables, median (IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset–diagnosis interval (months)</td>
<td>12 (8–18)</td>
<td>8 (6–12)</td>
<td>11 (6–21)</td>
<td>0.22</td>
</tr>
<tr>
<td>Survival from onset (months), censored</td>
<td>39 (24–72)</td>
<td>32 (23–54)</td>
<td>43 (32–59)</td>
<td>0.79</td>
</tr>
<tr>
<td>Survival from onset (months), uncensored (date of death available)</td>
<td>25 (20–56)</td>
<td>24 (15–56)</td>
<td>39 (30–55)</td>
<td>0.08</td>
</tr>
<tr>
<td>Survival from diagnosis (months), censored</td>
<td>25 (11–50)</td>
<td>26 (12–29)</td>
<td>28 (19–40)</td>
<td>0.63</td>
</tr>
<tr>
<td>Survival from diagnosis (months), uncensored (date of death available)</td>
<td>14 (9–37)</td>
<td>13 (8–29)</td>
<td>28 (18–38)</td>
<td>0.74</td>
</tr>
<tr>
<td>Nominal variables (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>38 (51)</td>
<td>7 (50)</td>
<td>17 (65)</td>
<td>0.44</td>
</tr>
<tr>
<td>Site of onset (spinal)</td>
<td>40 (54)</td>
<td>9 (64)</td>
<td>20 (77)</td>
<td>0.12</td>
</tr>
<tr>
<td>Familial ALS present (yes)</td>
<td>16 (22)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>0.06</td>
</tr>
<tr>
<td>Riluzole (prescribed)</td>
<td>29 (41)</td>
<td>2 (14)</td>
<td>10 (39)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 3 Known amyotrophic lateral sclerosis-associated variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant</th>
<th>Patient frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG</td>
<td>c.250A&gt;G (p.Lys84Glu)</td>
<td>1/115</td>
</tr>
<tr>
<td>C9orf72</td>
<td>Repeat expansion</td>
<td>2/115</td>
</tr>
<tr>
<td>CHCHD10</td>
<td>c.100C&gt;T (p.Pro33Ser)</td>
<td>1/115</td>
</tr>
<tr>
<td>DCTN1</td>
<td>c.2353C&gt;T (p.Arg785Trp)</td>
<td>2/115</td>
</tr>
<tr>
<td>DCTN1</td>
<td>c.3746C&gt;T (p.Thr1249Ile)</td>
<td>1/115</td>
</tr>
</tbody>
</table>

Table 4 Age of onset and survival periods for Hispanic/Latino clinic-based populations

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>Age of onset (years) Mean (95% CI)</th>
<th>Survival interval (months) Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monterrey, Mexico (2010)</td>
<td>61</td>
<td>47.5 (44.8 to 50.2)</td>
<td>63.0 (49.5 to 76.5)*</td>
</tr>
<tr>
<td>Havana, Cuba (2017)</td>
<td>115</td>
<td>53.0 (50.5 to 55.6)</td>
<td>39.0 (30.6 to 47.4)*</td>
</tr>
<tr>
<td>Rio de Janeiro, Brazil (2007)</td>
<td>227</td>
<td>53.6 (41.5 to 65.7)</td>
<td>49.0 (42.5 to 55.5)*</td>
</tr>
<tr>
<td>Montevideo, Uruguay (2017)</td>
<td>220</td>
<td>58.2 (56.5 to 60.0)</td>
<td>36.9 (31.6 to 42.3)*</td>
</tr>
<tr>
<td>Dublin, Ireland (2017)</td>
<td>1038</td>
<td>61.6 (60.9 to 62.4)</td>
<td>35.0 (32.6 to 37.4)*</td>
</tr>
<tr>
<td>Barcelona, Spain (2001)</td>
<td>215</td>
<td>64.3 (29.0 to 91.0)</td>
<td>21.0 (19.7 to 22.3)†</td>
</tr>
</tbody>
</table>

*Survival from onset. †Survival from diagnosis. ‡Median age of onset (range). §LAENALS study comparator population. ¶Population-based study.
at death within the cohort mirrored that of the population-based mortality study, and the proportion of patients within each ancestral population was also broadly reflective of that identified by the mortality study.

The study was also limited by the absence of genetic information available for the Uruguayan population. A more detailed genomic analysis of the Cuban population was limited by available material. Targeted sequencing was performed considering only known genes reliably linked to ALS or dementia, and it was not possible to comment on the existence of as-of-yet unknown genes of large effect or other potential ALS risk alleles within the Cuban population. Although we could not assess the degree of genetic admixture using available samples, previous studies have shown that within Cuba and Uruguay, there is evidence of variance in the extent of genetic admixture by geographical regions. Within Havana and its surrounds, between 65% and 84% of the population are identifiable as of European ancestry through the use of genetic markers, with between 11% and 24% and between 5% and 11% identifiable as of African and Native American ancestry, respectively.40 Furthermore, while the vast of the Montevideo population are of European ancestry, approximately 8% are of either African or Native American ancestry.41

Exploratory analysis in the Cuban study cohort revealed no difference in clinical or demographic variables between ethnicities, although interpretation of this is limited by the small numbers in each subcohort and by the absence of characterisation of respiratory and cognitive onset ALS in overall study cohort. Potential phenotypic differences could act as hidden covariants between different population groups and are important factors to account for when assessing differences in mortality rates in admixed populations. Indeed, the European ALS (EURALS) consortium, in a study of six population-based registries over a 2-year period, has previously demonstrated the existence of differences in type of onset by ethnicity across Europe.42 Larger scale, population-based, genome–phenotype correlation studies such as these are required to fully assess the impact of genetic admixture in Latin-American populations. The Latin-American Epidemiological Network of ALS study will perform this task in both the Cuban and Uruguayan populations, along with assessing the admixed Chilean population. This study will also aim to address limitations in characterisation of phenotype, particularly the cognitive aspects of ALS, by training all involved in appropriate standardised cognitive assessment techniques. Additional clinical information including disease progression and environmental exposures will be captured. Finally, direct genome-phenotype correlations will be optimised through the use of ancestral markers alongside self-reported ethnicity.

CONCLUSION

Our study describes for the first time the clinical characteristics of ALS in two understudied populations, Cuba and Uruguay, and has identified differences in age of onset/diagnosis between these populations and their Irish counterparts. Furthermore, we have demonstrated differences in the population genetics of ALS between the Cuban and Irish cohorts, with a significantly lower rate of C9orf72 in the former. Given the relatively high proportion of familial ALS in the Cuban population, the paucity of genetically identifiable causes of ALS suggests there are potentially large effect genes still to be discovered in this population. Finally, to further our understanding of the clinical impact of genetic admixture as regards ALS risk, larger scale, genome-phenotype correlation studies based on multicentre international collaboration are required and should focus on relatively less well studied populations such as those in the Caribbean and South America.

Contributors MR: study concept and design, analysis and interpretation of data, manuscript composition. TZV: study concept and design, acquisition of data, analysis and interpretation of data, revision of manuscript for intellectual content. RLM, MAD: acquisition of data, analysis and interpretation of data. JR, MH: study concept and design, acquisition of data, analysis and interpretation of data, revision of manuscript for intellectual content. JG, GEL-F, HH, MCV: study concept and design, acquisition of data. MMP: study concept and design, analysis and interpretation of data. AP: study concept and design, acquisition of data, analysis and interpretation of data. MM, CNK: study concept and design. GL: study concept and design, analysis and interpretation of data, revision of manuscript for intellectual content. MR: statistical analysis.

Funding This project is the work of the Latin-American Epidemiology Network of ALS (LAENALS), supported through funding provided by the Centers for Disease Control and Prevention (Award T5000242). MR and MAD receive support from Science Foundation Ireland. TZV, IG, GEL-F and MMP receive support from the Ministry of Public Health Cuba. RLM receives support from the Motor Neurone Disease Association (MND). OH receives support from the Health Research Board including Joint Programme in Neurodegeneration, Research Motor Neurone and Science Foundation Ireland (FutureNeuro Centre).

Competing interests OH has received speaker honoraria/travel funding from Janssen Cilag, Biogen Idec, Sanofi Aventis, Novartis and Merck-Serono; has been a member of advisory panels for Biogen Idec, Allergan, Ono Pharmaceutical, Novartis, Cytokinetics, Treeway, Wave, NINDS CDE Team for ALS/MD and Sanofi Aventis; serves as Editor-in-Chief of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia; serves on the editorial board of the Journal of Neurology, Neurosurgery, and Psychiatry; coholds patents for Treatment of Central Nervous System Injury Inventors (RCSI); consults for Biogen Idec and Cytokinetics; and has received research support from Science Foundation Ireland.

Patient consent for publication Not required.

Ethics approval Informed written consent was obtained from all participants, and the study was approved by the research and ethics committees of all participating institutes.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data can be obtained on request through the LAENALS management team (ryanm65@ctd.ie), which has a protocol for approving data requests. Data cannot be made publicly available because of restrictions based on informed consent of the participants.

REFERENCES

Reduced frequency of s: 1–7. doi:10.1136/jnnp-2018-319838.
Determining the incidence of familiality in ALS
A study of temporal trends in Ireland from 1994 to 2016

Marie Ryan, MRCPI, Mark Heverin, MSc, Mark A. Doherty, BSc, Nicola Davis, MRes, Emma M. Corr, PhD, Alice Vajda, PhD, Niall Pender, PhD, Russell McLaughlin, PhD, and Orla Hardiman, MD

Neurol Genet 2018;4:e239. doi:10.1212/NXG.0000000000000239

Abstract
Objective
To assess temporal trends in familial amyotrophic lateral sclerosis (FALS) incidence rates in an Irish population and to determine factors influencing FALS ascertainment.

Methods
Population-based data collected over 23 years, using the Irish amyotrophic lateral sclerosis (ALS) register and DNA biobank, were analyzed and age-standardized rates of FALS and associated familial neuropsychiatric endophenotypes were identified.

Results
Between 1994 and 2016, 269 patients with a family history of ALS from 197 unique families were included on the register. Using stringent diagnostic criteria for FALS, the mean age-standardized FALS incidence rate for the study period was 11.1% (95% confidence interval [CI], 8.8–13.4). The FALS incidence rate increased steadily from 5.2% in 1994 to 19.1% in 2016, an annual increase of 0.7% (95% CI, 0.5–0.9, p < 0.0001). Inclusion of the presence of neuropsychiatric endophenotypes within kindreds increased the FALS incidence rate to 30%. The incidence of FALS in newly diagnosed individuals from known families increased significantly with time, accounting for 50% of all FALS diagnoses by 2016. The mean annual rate of recategorization from "sporadic ALS" to "FALS" was 3% (95% CI, 2.6–3.8).

Conclusions
The true population-based rate of FALS is at least 20%. Inclusion of extended endophenotypes within kindreds increases the rate of FALS to 30%. Cross-sectional analysis of clinic-based cohorts and stringent definitions of FALS underestimate the true rate of familial disease. This has implications for genetic counseling and in the recognition of presymptomatic stages of ALS.
Glossary

ALS = amyotrophic lateral sclerosis; CI = confidence interval; FALS = familial ALS; FTD = frontotemporal lobar dementia; SALS = sporadic ALS.

Amyotrophic lateral sclerosis (ALS) is recognized as a primary motor system degeneration of complex genetic origin. The condition is usually distinguished into “familial ALS” (FALS), in which other family members are reported to have had ALS, and “sporadic ALS” (SALS), in which there is no discernible family history. At least 30 mendelian-inherited genes have been implicated in the familial form of ALS.1 However, variants in the majority of mendelian-inherited genes have also been identified in apparently sporadic cases.2–5

Recent modeling suggests that the interaction between the genetic and environmental risk factors varies depending on the specific genetic variants involved, implying that most genetic causes of ALS are likely to be incompletely penetrant, and that most apparently, SALS occurs in the context of variable genomic risk.6,7 Moreover, higher rates of neuropsychiatric conditions have been described among first- and second-degree relatives of ALS probands,8,9 and 14% psychiatric conditions have been described among elsewhere.11,12 Briefly, individuals confirmed to have possible, probable, or definite ALS according to the El Escorial criteria14 are enrolled on the register. Following confirmation of diagnosis, a semistructured telephone interview is conducted using a standardized questionnaire. All informants are asked specifically about the occurrence of any neurodegenerative or neuropsychiatric conditions among their first- and second-degree relatives. A diagnosis of frontotemporal lobar dementia (FTD) in a relative is accepted if (1) they were diagnosed by a physician with expertise in cognitive disorders or (2) the description of the relative was deemed to meet the Neary criteria15 by a neurologist experienced in diagnosing patients with FTD. For those identified posthumously, a direct chart review and interview with a family member is conducted where possible. The ALS DNA biobank has been used to identify established pathogenic variants in genes implicated in ALS pathogenesis.16 These DNA samples are stored to allow for retrospective assessment of new genes.

Standard protocol approvals, registrations, and patient consents

All patients included in the Irish ALS register between January 1, 1994, and December 31, 2016, were invited to participate in the study. Informed written consent for the study was obtained from all participants. This study was approved by the Beaumont Hospital Research Ethics Committee (15/40).

Diagnostic criteria for FALS

The presence and classification of FALS was determined using our previously defined criteria (figure 1).17

Analysis of register data

Data from the Irish ALS register from 1994 to 2016 were interrogated. All cases reporting a history of suspected or confirmed ALS or FTD in at least 1 relative were collated. Where necessary, genealogical records were reviewed. The DNA database was cross-referenced with the clinical database, and the genetic status was determined in all cases for whom DNA was available. Additional individuals with a known gene variant, not previously identified by a family history of ALS or FTD, were collated. Cases with a diagnosis of Kennedy disease were excluded. Individuals who had a family history suspicious for ALS (e.g., relative died of “muscle wasting disease”), in whom we could not confirm the diagnosis, were excluded. Similarly, individuals with a relative with dementia, in whom the nature of the dementia could not be accurately determined, were excluded.

Our previously reported criteria were applied to all identified FALS cases. Annual age-standardized incidence rates for FALS

Methods

Data collection

An Irish population-based register for patients with ALS has been in operation since 1994 with an associated DNA bank operating since 1999.11–13 Details of case ascertainment and validation methodologies have been described extensively elsewhere.11,12 Briefly, individuals confirmed to have possible, probable, or definite ALS according to the El Escorial criteria are enrolled on the register. Following confirmation of diagnosis, a semistructured telephone interview is conducted using a standardized questionnaire. All informants are asked specifically about the occurrence of any neurodegenerative or neuropsychiatric conditions among their first- and second-degree relatives. A diagnosis of frontotemporal lobar dementia (FTD) in a relative is accepted if (1) they were diagnosed by a physician with expertise in cognitive disorders or (2) the description of the relative was deemed to meet the Neary criteria by a neurologist experienced in diagnosing patients with FTD. For those identified posthumously, a direct chart review and interview with a family member is conducted where possible. The ALS DNA biobank has been used to identify established pathogenic variants in genes implicated in ALS pathogenesis. These DNA samples are stored to allow for retrospective assessment of new genes.

Figure 1 Byrne criteria for familial amyotrophic lateral sclerosis (FALS)

| Definite FALS: | A patient with ALS with at least two first- or second-degree relatives with ALS OR a patient with ALS with at least one relative with ALS and gene-positive cosegregation. |
| Probable FALS: | A patient with ALS with one first- or second-degree relative with ALS. |
| Possible FALS: | A patient with ALS with a distant relative with ALS OR a patient with sporadic ALS, but positive for a FALS gene OR a patient with ALS with a family member with confirmed frontotemporal dementia. |
and FALS subclassifications were calculated as a proportion of the total number of ALS cases diagnosed annually, using the pooled Irish ALS register population from 1994 to 2016 and considering the following age bands: ≤39, 40–49, 50–59, 60–69, 70–79 and 80+ years.

Where multiple members of the same kindred were identified, pedigrees were constructed to include first-, second-, and third-degree relatives where possible. All newly diagnosed individuals from previously identified kindreds were identified. Crude incidence rates of newly diagnosed individuals from known families were calculated as a proportion of total number of ALS families presenting annually. Dates of diagnosis of FALS for all cases were obtained from the ALS register and cross-referenced against medical records where applicable. All cases were grouped by whether they were recategorized from SALS to FALS or identified as FALS at the time of diagnosis. Annual rates of recategorization were calculated by dividing the number of recategorized FALS individuals by the number of individuals diagnosed with ALS annually.

Analysis by additional phenotype and endophenotype
All cases with a confirmed family history of FTD were identified. Secondary analysis identified all probands with a confirmed family history of all-type dementia and/or schizophrenia/psychosis. The crude incidence rates of probands with a confirmed family history of FTD, all-type dementia, and possible familial endophenotypes (e.g., schizophrenia/psychosis) were calculated by dividing the number of patients diagnosed with ALS annually.

Genetic screening and analysis
All patients were tested for established high-penetration ALS-associated variants. Patients were screened for the presence of the pathogenic GGGGCC hexanucleotide repeat expansion in C9orf72 by repeat-primed PCR as described previously. This methodology has previously been validated with positive and negative controls using reverse transcriptase PCR and Southern blots. Amplified fragments were measured by capillary electrophoresis on an Applied Biosystems 3500 Series Genetic Analyzer and visualized using Gene Mapper v.4.0. Patients with 30 hexanucleotide repeats or above were deemed positive for the expansion. Next-generation DNA sequencing was performed through Illumina paired-end, PCR-free, whole-genome sequencing and Illumina paired-end, target-enriched sequencing. Sequence data were generated for 676 patients and 446 population-matched controls. Participants were screened for exonic and splice-site variants in the exons of 30 genes considered to be linked to ALS (ALS2, ANG, CHCHD10, CHMP2B, DAO, DCTN1, ELP3, ERBB4, FIG4, FUS, hnRNPA1, LMBN1, MATR3, NEFH, OPTN, PFN1, PRPH, SETH, SIGMARI, SOD1, SPAST, SPG11, SQSTM1, TAF15, TARDBP, TBK1, UBQLN2, UNC13A, VAPB, and VCP). To screen for high penetrance, variants that were present in any controls or at a maximum allele frequency exceeding 0.05 in reference population data sets were filtered. Variants that were present in the ALSoD (ALSonline genetics database) or the Human Gene Mutation Database V.2017 and are reported in the literature as being familial or highly penetrant were considered to be mendelian causes of ALS. Patients for whom DNA was not available and without confirmed family history of ALS or FTD were categorized as nonfamilial cases. All cases with an established mendelian-inherited ALS gene variant were identified, and the crude incidence rate of mendelian-inherited ALS was calculated by dividing by the number of patients diagnosed with ALS annually.

Statistical analysis
We fitted linear regression models to estimate the annual mean change in incidence rates with calendar year as the predictor variable and dependent variables: total FALS, definite FALS, probable FALS, possible FALS, FALS from previously identified families, recategorized FALS, mendelian-inherited ALS and probands with a positive family history of FTD, all-type dementia, and schizophrenia/psychosis, respectively. To determine whether increasing rates of FALS were a function of higher rates of “possible FALS” diagnoses, we tested the hypothesis that the total FALS (b1) and combined “definite” and “probable FALS” (b2) beta coefficients were not statistically different from each other. A simple linear regression model with total FALS and combined “definite” and “probable FALS” as main effects with joint interaction term (b1*b2) was fitted via bias corrected bootstrap (1,000 resamples). SPSS Statistics Version 24 was used to identify and estimate the parameters of the linear models and to test for statistical significance.

Results
Demographics
A total of 2173 individuals, diagnosed with ALS between 1994 and 2016, were recorded on the Irish ALS register. Of these, 313 individuals had potential FALS based on a family history of suspected or confirmed ALS or FTD in at least 1 relative or the presence of the mendelian-inherited ALS gene mutation in the proband (figure 2A). Thirty-five individuals with a family history suspicious for ALS or FTD in whom it was not possible to confirm the relatives’ diagnoses and 9 individuals with proven Kennedy disease were excluded. Two hundred sixty-nine registered patients with ALS comprising 197 unique families were included in the final analysis (figure 2B). Ninety-four individuals carried a known ALS-causative gene mutation (C9orf72, TARDBP, FUS, SOD1, and SQSTM1). Fifty-one patients carrying the C9orf72 variant reported a family history of FTD (figure e1, links.lww.com/NXG/A49).

Secondary analysis of the Irish ALS register identified 392 patients with a confirmed relative with dementia (reported as Alzheimer disease [131], FTD [51], and unspecified [210]) and 57 patients with a confirmed family history of schizophrenia.
Incidence of FALS

The mean annual crude FALS incidence rate was 11.1% (95% confidence interval [CI], 8.9–13.3) for the study period, and the corresponding mean age-standardized FALS incidence rate was 11.1% (95% CI, 8.8–13.4). However, the age-standardized FALS incidence rate increased steadily from 5.2% in 1994 to 19.1% in 2016, representing an overall increase of 0.7% (95% CI, 0.5–0.9, p < .0001) per annum (figure 3A). Using our previously published criteria for “definite FALS,” the mean age-standardized incidence rate was 4% (95% CI, 2.9–5.0) for the entire study period. However, between 1994 and 2016, this increased at a rate of 0.2% (95% CI, 0.01–0.3, p = 0.007) annually (figure 3B). For “probable FALS,” the age-standardized incidence rate was 3.1% (95% CI, 2.3–3.9) but did not increase with time (p = 0.318) (figure 3C). Conversely, the age-standardized incidence for “possible FALS” increased by 0.4% (95% CI, 0.2–0.5, p < 0.0001) annually, with an overall mean rate of 4.6% (95% CI, 3.1–6.1) (figure 3D). There was no difference between the total FALS (b = 0.007) and combined “definite” and “probable FALS” (b = 0.003) beta coefficients (p = 0.671) (figure 3E).

FALS from previously identified families and recategorized FALS

A mean of 2.9% (95% CI, 1.8–4.1) of individuals diagnosed with ALS each year were from known FALS families, increasing by 0.3% annually (95% CI, 0.2–0.4, p < 0.0001). The relative contribution of newly diagnosed individuals from known families increased annually (p = 0.001), accounting for 50% of all FALS diagnoses in 2016. To prevent an underestimation of effect size due to unrecognized FALS in SALS individuals (i.e., those in whom a second family member has not yet been affected), the final 3 years of data collection were excluded. For the remaining years, the overall mean rate of recategorization from sporadic to FALS was 3% (95% CI, 2.6–3.8) annually. This did not change with time (p = 0.177).

Mendelian-inherited ALS

From 1999 to 2016, the mean crude incidence rate for known mendelian-inherited ALS was 4.49% (95% CI, 2.8–6.2) annually. A temporal increase of 0.4% (95% CI, 0.2–0.6) annually (p = 0.02) was observed, driven by C9orf72-positive ALS patients, who accounted for 89 of 94 known mendelian-inherited forms. The other cases, all of which were sporadic, were associated with mutations in TARDBP (1), FUS (2), SQSTM1 (1), and a previously recognized rare SOD1 variant.

Impact of phenotype

From 1994 to 2016, a mean of 1.9% (95% CI, 1.1–2.9) of individuals diagnosed with ALS annually had a confirmed positive family history of FTD, increasing by 0.2% (95% CI, 0.01–0.39; p = 0.001) per year. The mean crude incidence rates of probands with a confirmed family history of any form of dementia or schizophrenia were 14.87% (95% CI, 9.3–20.5) and 2.2% (95% CI, 1.4–3.0), respectively. Both demonstrated annual increases of 1.8% (95% CI, 1.4–2.2; p = 0.0001) and 0.2% (95% CI, 0.18–0.22; p = 0.0001), respectively.

Discussion

The Irish ALS population-based register is flexibly designed to allow for both the categorization of FALS and the application of different levels of stringency of the definition of FALS. The availability of data from the register, combined with island status, low immigration rates, and comparatively large family sizes makes Ireland an ideal place to study FALS. By applying our previously reported criteria to the Irish ALS population-based register, we have shown that the mean annual age-standardized incidence rate for any definition of FALS over a 23-year period was 11.1%. This is consistent with the commonly quoted figure of 10% for the population rate of FALS observed in other populations of European ancestry.21,22 However, we have also identified an increasing trend in FALS ascertainment, ranging from 5.2% in 1994 to 19.1% in 2016, with over 50% of newly diagnosed FALS cases in 2016 coming from second generations within known FALS families. This in turn may drive the observed increasing trend in “definite” FALS as with increasing awareness of the prevalent FALS families, certainty of FALS classification increases.
The mean rate of recategorization from “SALS” to “FALS” was 3% annually. This is higher than previously reported crude incidence rates of ALS in relatives of patients with SALS of 1.2%. The difference is most likely a function of differences in classification criteria used. Indeed, there are numerous reasons why some FALS individuals are misclassified as SALS, including incomplete ascertainment of extended kindreds, and small family size. Less frequently, SALS may be misclassified as FALS. The methodological approach used in this study is consistent with standard approaches used in a clinical environment with only ALS cases with confirmed ALS and/or FTD cases in relatives included in our analysis. Overall, as the opportunities to misclassify FALS as SALS are greater than vice versa, the possibility of underestimating the true FALS rate within this study far exceeds the possibility an overestimation.

We have also found a mean incidence rate of known mendelian-inherited genes associated with ALS of 4.49% (95% CI, 2.8–6.2), increasing annually. This figure is driven by C9orf72-positive ALS, which accounts for the majority of known mendelian-inherited ALS in Ireland. In our data set, of the 35 C9orf72-positive ALS patients without a confirmed family history of ALS or FTD, 9 patients were unable to provide complete information on their relatives. The remaining patients reported at least 1 first- or second-degree relative with unspecified dementia, a neuropsychiatric disorder or unconfirmed ALS (figure e-2, links.lww.com/NXG/A49). Our findings are thus in keeping with our previous work suggesting that truly “sporadic” ALS associated with C9orf72 repeat expansions are rare and that the majority of those carrying this variant have a family history of either neurodegenerative or neuropsychiatric disease.

We have also demonstrated an increased trend in incidence of probands with confirmed positive family histories of FTD, all-type dementia, and schizophrenia reflecting the recognition of the importance of the extended phenotype within ALS kindreds. Our findings also demonstrate a systematic difference in the clinical phenotype between historical cases and those recently enrolled consistent with information creep, a common finding in longer standing registers. Our observations strongly suggest that FALS criteria should be expanded to
incorporate the presence of these extended phenotypes within ALS kindreds. We have previously shown that the likelihood of FALS increases based on the number of patients with ALS within a kindred and the size of the extended kindred. Using the same analyses, we have now calculated that a kindred of 38 will introduce a 5% probability of having a relative with FTD by chance alone, but that the presence of 2 or more relatives with FTD is sufficiently unlikely by chance (p = 0.025) to provide a credible criterion for FALS. Similarly, we have calculated a 5% probability of having 1 relative with schizophrenia in the presence of at least 5 unaffected relatives. However, there is a diminishing probability of having 2 or more first- or second-degree relatives with schizophrenia within extended kindreds, rendering the presence of 3 or more family members with schizophrenia, a credible criterion by which to extend the definition of FALS (figure 4A). This calculation is consistent with our recent cluster analysis of ALS kindreds with extended neuropsychiatric endophenotypes. Conversely, assuming a lifetime risk of developing dementia in those over 65 of approximately 1 in 5, the presence of dementia in 2 relatives within an ALS kindred is insufficient to make a diagnosis of likely FALS, irrespective of the family size (figure 4B).

Using stringent diagnostic criteria and excluding the presence of a neuropsychiatric endophenotype within kindreds, our data suggest that at least 20% of ALS cases in Ireland have FALS. Of these, over 40% of Irish ALS families carry the C9orf72 repeat expansion. However, by including a wider neuropsychiatric endophenotype among relatives as an additional criterion, the rate of FALS is likely to be in the region of 30%, although definitive estimates cannot yet be generated using our population-based register data, as historical ascertainment is incomplete.

Figure 4 Binomial probability distribution

For the number of relatives with schizophrenia (A) or dementia (B) against the kindred size. p < 0.05 is highlighted in blue.
This study has limitations. The presence of the C9orf72 repeat expansion was determined by repeat-primed PCR plus amplicon length analysis in blood samples. Although confirmation of each repeat expansion length using Southern blotting is recommended, and although there is acknowledged variation in amplicon length across the tissues, the approach used in this study was validated using positive and negative controls confirmed using Southern blot and is consistent with that adopted in the setting of diagnostic screening.

The definition and utility of the concept of FALS remains a matter of debate. Our data demonstrate that the estimated frequency of FALS within a population can be biased by both ascertainment methods, the level of stringency applied to the definition, and the inclusion or exclusion of extended phenotypes and endophenotypes that are biologically associated with ALS. Our population-based longitudinal data indicate that at least 20% of ALS is familial using stringent criteria. However, our data also suggest that a wider diagnostic categorization, to include FTD and neuropsychiatric conditions, is warranted.

Author contributions
M. Ryan: study concept and design, analysis and interpretation of data, and manuscript composition. M. Heverin: study concept and design, analysis and interpretation of data, and revision of the manuscript for intellectual content. M.A. Doherty: acquisition of data, analysis and interpretation of data, and revision of the manuscript for intellectual content. N. Davis and E.M. Corr: acquisition of data. A. Vajda and N. Pender: study concept and design. R. McLaughlin: analysis and interpretation of data and revision of the manuscript for intellectual content. O. Hardiman: study concept and design, analysis and interpretation of data, and revision of the manuscript for intellectual content.

Study funding
The project is supported through the funding provided by Science Foundation Ireland (15/SPP/3244 and 16/ERC/3854). O.H. receives support from the Health Research Board including Joint Programme in Neurodegeneration, Research Motor Neurone, and Science Foundation Ireland (Future-eNeuro Centre). R.L.M. receives support from the Motor Neurone Disease Association (MND).

Disclosure
M. Ryan has received research support from Science Foundation Ireland. M. Heverin, M.A. Doherty, N. Davis, and E.M. Corr report no disclosures. A. Vajda has received research support from the Irish Health Research Board. N. Pender has received a speaker honorarium from Biogen; has been a member of advisory panels for Biogen Idec, Allergan, Ono Pharmaceuticals, Novartis, Cytokinetics, Treeway, Wave, NINDS CDE Team for ALS/MND, and Sanofi Aventis; serves as Editor-in-Chief of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia; serves on the editorial board of the Journal of Neurology Neurosurgery; coholds patents for Treatment of Central Nervous System Injury Inventors (RCSI); consults for Biogen Idec and Cytokinetics; and has received research support from Science Foundation Ireland. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Received February 6, 2018. Accepted in final form April 6, 2018.

References