Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.
The Clinical Characterisation and Genetic Epidemiology of Familial ALS in Ireland

PhD Thesis
Trinity College, 2012

Susan Byrne MB BAO BCh MRCPI
Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work. I agree to deposit this thesis in the University's open access institutional repository or allow the college to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Signed

[Signature]

Susan Byrne  Student number 09128476

Date 18/06/2013
Acknowledgements

The research detailed herein was carried out with the support of the Irish Health Research Board and Research Motor Neuron.

I would like to thank all of the patients with ALS and their families for the generous donation of their time, for sharing stories, and for imparting details of their family history with great care and attention.

I would like to thank my supervisor, Professor Orla Hardiman, for asking me to join her team, for teaching me about all aspects of ALS, and for her dedication to finding a cure for ALS.

I am greatly indebted to my past and present colleagues: Mark Heverin, Catherine Lynch, Marwa Elamin, Peter Bede, Cathal Walsh, Russell McLaughlin, Kevin Kenna, Bernie Corr, and Norah Jordan, especially for their willingness to tease out new theories and for their enthusiasm for research.

I owe unending thanks to my wonderful friends, especially Iain Jordan, and my amazing family, Claire, Jim, Kevin, and David, for their unwavering support, now and always.
Thesis Summary

The overall objective of this thesis is to collate the existing literature on familial ALS and to address shortcomings in the research, to investigate aggregation of ALS and other conditions among relatives of patients with ALS, to characterise the C9orf72 phenotype, and to identify possible candidates for future genetic studies. This is done through a comprehensive epidemiological characterisation of the Irish ALS population with a particular focus on familial ALS, and through a detailed family aggregation study of ALS patients and their relatives.

The Irish ALS register is used to characterise the Irish ALS population with the aim of identifying subgroups of patients with familial disease who may share individual genes. A detailed chapter describes the phenotype associated with the C9orf72 repeat expansion.

Results from this thesis include estimation of the rate of FALS; through a systematic review and meta-analysis, the population-based rate is estimated to be 5.1%, however this is subsequently refuted by the findings from the family aggregation study, containing almost 10,000 relatives of ALS patients and controls, which demonstrates that the rate of familial ALS is closer to 16%. Assessment of the risk of developing ALS estimates that relatives of ALS patients with the C9orf72 repeat expansion have a HR of 34, and relatives of ALS patients without the C9orf72 repeat expansion have a HR of 2, compared to relatives of controls.

The aggregation of schizophrenia and suicide is also elevated in the relatives of ALS patients compared to relatives of controls.

In the final section, findings generated from this thesis are used to propose future research projects, and candidates for future genetic studies are considered.
Data acquisition for this thesis

- Family projects, family aggregation study, and family aggregation databases were designed by Susan Byrne. Data for the family aggregation study was collected by Susan Byrne and Mark Heverin.
- The $\text{C9orf72}$ project was initiated by Susan Byrne, who tested the DNA samples, analysed the data, and designed the algorithm.
- Epidemiological data were extracted from the Register, and prepared for analysis by Susan Byrne.
- Susan Byrne performed all of the statistical data analysis.
- Data collected for the longitudinal study of ALS and cognition were collected between 2006 and 2012 by Dr. Marwa Elamin, Dr. Peter Bede, Dr. Julie Phukan, and Dr. Norah Jordan.
- Imaging data were collected and analysed by Dr. Peter Bede.
- Genetic testing for known genes was carried out by Dr. Russell McLaughlin and Mr. Kevin Kenna.
# Table of Contents

## CHAPTER 1  INTRODUCTION TO AMYOTROPHIC LATERAL SCLEROSIS .......... 27

1.1 Clinical presentation .......................................................................................................................... 27
   1.1.1 Motor symptoms............................................................................................................................... 27
   1.1.2 Cognition in ALS patients ............................................................................................................. 27
   1.1.3 Treatment options in ALS .............................................................................................................. 29

1.2 Epidemiology of ALS ..................................................................................................................... 30
   1.2.1 Incidence of ALS ............................................................................................................................ 30
   1.2.2 Gender................................................................................................................................................ 30
   1.2.3 Age at disease onset .......................................................................................................................... 30
   1.2.4 Survival................................................................................................................................................ 31
   1.2.5 Phenotype and genotype correlation ................................................................................................. 32
   1.2.6 Benefit of prospective population-based ALS Registers ................................................................. 32

1.3 Familial ALS..................................................................................................................................... 34
   1.3.1 Defining familial ALS........................................................................................................................ 34
   1.3.2 Rate of familial ALS ........................................................................................................................... 35
   1.3.3 Risk of other family members developing ALS .................................................................................. 36

1.4 Genetics in ALS .............................................................................................................................. 40
   1.4.1 Single-gene causes of ALS ................................................................................................................ 40
   1.4.2 Oligogenic theory ............................................................................................................................. 41
   1.4.3 Complex genetics of Sporadic ALS ................................................................................................. 42
   1.4.4 Methods of gene discovery in ALS ................................................................................................. 42
   1.4.5 Linkage analysis .................................................................................................................................. 43
   1.4.6 Genome-wide associations studies ................................................................................................. 45
   1.4.7 Candidate gene approach .................................................................................................................. 46
   1.4.8 Exome Sequencing ........................................................................................................................... 47
1.4.9 Gene Penetrance .......................................................................................................................... 49

1.5 Discovery of the C9orf72 repeat expansion in ALS ........................................................................ 51

1.6 Genetic counseling in ALS .............................................................................................................. 53

1.7 Family aggregation studies in ALS .................................................................................................. 55
  1.7.1 Introduction to family aggregation ............................................................................................... 55
  1.7.2 Family aggregation studies in ALS ............................................................................................... 56
  1.7.3 Single case reports of aggregation of other conditions in patients with ALS and their family members .......................................................................................................................................................................................... 62
  1.7.4 Aggregation of other psychiatric conditions in families .................................................................. 64
  1.7.5 Methodology used in family aggregation studies ........................................................................... 65
  1.7.6 Analytical methods used to describe the results from family aggregation studies ..................... 67

1.8 Common themes in neurodegenerative and neuropsychiatric disease ........................................... 70
  1.8.1 Introduction ..................................................................................................................................... 70
  1.8.2 Common themes: Clinical features of neurodegenerative disease ............................................... 70
  1.8.3 Common themes: Neuropathology ................................................................................................. 72
  1.8.4 Common themes: Genetics ............................................................................................................. 74
  1.8.5 Common themes: Biochemical processes ..................................................................................... 76

1.9 Summary .......................................................................................................................................... 85

CHAPTER 2 AIMS ....................................................................................................................................... 87

2.1 Familial Amyotrophic Lateral Sclerosis ............................................................................................. 87

2.2 Family aggregation study ................................................................................................................. 87

2.3 Utilization of epidemiological information to identify subgroups for genetic analysis and characterise phenotypes .................................................................................................................................................................................. 89
  2.3.1 Description of ALS epidemiology in a population based register ................................................. 89
  2.3.2 Characterization of C9orf72 phenotype ......................................................................................... 90
CHAPTER 3  FAMILIAL ALS

3.1 Study A: To determine if there is consensus among clinicians on the definition of the term Familial Amyotrophic Lateral Sclerosis

3.1.1 Introduction
3.1.2 Aims
3.1.3 Method
3.1.4 Results
3.1.5 Discussion
3.1.6 Proposing criteria for Familial ALS
3.1.7 Conclusion

3.2 Study B: To report the rate of Familial Amyotrophic Lateral Sclerosis using systematic review and meta-analysis methodology

3.2.1 Introduction: rate of familial ALS
3.2.2 Aims
3.2.3 Methods
3.2.4 Results
3.2.5 Discussion
3.2.6 Conclusion

CHAPTER 4  EPIDEMIOLOGY OF ALS

4.1 General introduction

4.2 Study A: Epidemiology of ALS in the island of Ireland; 1996-2011

4.2.1 Description of the Irish ALS Register
4.2.2 Aims
4.2.3 Methods
4.2.4 Results: Capture-Recapture
4.2.5 Results: General demographics
4.2.6 Results: Site of onset and gender distribution
4.2.7 Results: Age at symptom onset and diagnosis of ALS
4.2.8 Results: Proportion of cases diagnosed in each age category.......................................124
4.2.9 Results: Incidence................................................................................................................ 126
4.2.10 Results: Prevalence............................................................................................................127
4.2.11 Results: Use of intervention.............................................................................................127
4.2.12 Results: Survival in ALS between time cohorts............................................................128
4.2.13 Results: Interventions and attendance at specialist clinics ...........................................129
4.2.14 Results: Survival in sporadic onset versus familial onset ALS cases........................130
4.2.15 Results: Survival in ALS by age at disease diagnosis ...................................................131
4.2.16 Results: Identifying subgroups within the familial ALS cohort....................................132
4.2.17 Results: Geographic location at time of diagnosis........................................................134
4.2.18 Results: Familial cases per county...................................................................................135
4.2.19 Results: Surnames on the register...................................................................................136

4.3 Comparison of ALS epidemiology in Ireland and Northern, 2005-2010 ..................137
4.3.1 Results: General demographics...........................................................................................137
4.3.2 Results: Incidence..................................................................................................................138
4.3.3 Results: Prevalence...............................................................................................................139
4.3.4 Results: Survival....................................................................................................................140
4.3.5 Discussion...............................................................................................................................141

CHAPTER 5 A POPULATION-BASED COHORT STUDY TO DESCRIBE THE
PHENOTYPE OF PATIENTS WITH ALS CARRYING A C9ORF72
REPEAT EXPANSION.................................................................149

5.1 Introduction...............................................................................................................................149
5.2 Aims .........................................................................................................................................149
5.3 Methods.....................................................................................................................................149
5.3.1 Sample Selection....................................................................................................................149
5.3.2 Genetic testing hexanucleotide repeat expansion in C9orf72..........................................149
5.3.3 Mutation Screening for known genes................................................................................150
5.3.4 Population-based cohort of incident ALS cases (n=191)..................................................150
5.3.5 Imaging.............................................................................................................................................. 152
5.3.6 Statistical methods..............................................................................................................................153

5.4 Results............................................................................................................................................. 153
5.4.1 Genetic testing hexanucleotide repeat expansion in C9orf72 (n=435).........................................153
5.4.2 Population-based cohort of incident ALS cases (n=191)..............................................................156
5.4.3 Family history in patients with the C9orf72 repeat expansion....................................................156
5.4.4 Disease progression............................................................................................................................ 157
5.4.5 Cognitive profile.................................................................................................................................159
5.4.6 Cognitive profile of Patients with co-morbid frontotemporal dementia (ALS-FTD).................161
5.4.7 Behavioural profile.............................................................................................................................162
5.4.8 Imaging............................................................................................................................................ 163
5.4.9 Assessment of risk...............................................................................................................................165

5.5 Discussion..................................................................................................................................... 166
5.5.1 Conclusion.........................................................................................................................................171

CHAPTER 6 FAMILY AGGREGATION STUDY ................................................................. 173

6.1 Introduction................................................................................................................................ 173

6.2 Aims................................................................................................................................................. 173

6.3 Methods.......................................................................................................................................... 174
6.3.1 Study design....................................................................................................................................174
6.3.2 Power...............................................................................................................................................176
6.3.3 Data collection.................................................................................................................................176
6.3.4 Design of questionnaire....................................................................................................................176
6.3.5 Inclusion and exclusion criteria for recruitment and selection of participants..........................179
6.3.6 Family history data collection........................................................................................................179
6.3.7 Procedures for processing the family history questionnaire.......................................................180
6.3.8 Online resources for verifying family history..............................................................................182
6.3.9 Verification of reported cause of death with official death certificates ..........183
6.3.10 Database ...........................................................................................................184
6.3.11 Data storage and confidentiality .................................................................184
6.3.12 Statistical analysis .........................................................................................185

6.4 Results of Aggregation study: General .............................................................187
6.4.1 Recruitment of ALS patients ........................................................................187
6.4.2 Recruitment of controls ................................................................................189
6.4.3 Quality of data collected ................................................................................190
6.4.4 Demographic comparison between cases and controls ..............................190
6.4.5 Number of first- and second-degree relatives ...........................................191
6.4.6 Cause of death ...............................................................................................192
6.4.7 Verification of cause of death ........................................................................193

6.5 Results of Aggregation study: Familial ALS ....................................................194
6.5.1 Family aggregation study: Reported cases of familial ALS at study inception 194
6.5.2 Family aggregation study: Additional relatives found to have ALS through aggregation study .................................................................198
6.5.3 Population based rate of FALS ......................................................................203
6.5.4 Proportion of controls reporting a family history of ALS ............................203
6.5.5 Genetic testing in ALS patient participating in the study ............................203
6.5.6 Classification methods for FALS cases .........................................................204
6.5.7 Relative risk of ALS .......................................................................................204
6.5.8 Segregation analysis of FALS cases ..............................................................205
6.5.9 Informative families .....................................................................................207

6.6 Results of Aggregation study: Neurodegenerative and neuropsychiatric diseases

211
6.6.1 Analysis to look for effects of case clustering in families ..........................211
6.6.2 Initial analysis ...............................................................................................211
6.6.3 Subgroup analysis; stratifying for the C9orf72 repeat expansion ...............212
6.6.4 Subgroup analysis; Cognitive testing ...........................................................214
6.6.5 Subgroup analysis; age at onset of disease ...............................................215
6.7 Results of Aggregation study: Fecundity............................................................................. 216

6.8 Discussion................................................................................................................................... 219

6.8.1 General points................................................................................................................................. 219
6.8.2 Verification method............................................................................................................................... 219
6.8.3 Familial ALS ...................................................................................................................................... 222
6.8.4 Aggregation of Neurodegenerative and Neuropsychiatric diseases............................................. 224
6.8.5 Aggregation of other diseases ........................................................................................................ 229
6.8.6 Selection of candidates for exome sequencing project .............................................................. 230
6.8.7 Genetic counselling in FALS........................................................................................................ 231
6.8.8 Fecundity........................................................................................................................................... 232

6.9 Conclusion.................................................................................................................................... 233

CHAPTER 7 SUMMARY, DISCUSSION AND FURTHER WORK............................................ 235

7.1 Summary of findings.................................................................................................................. 235

7.1.1 Familial ALS and family aggregation study............................................................................ 235
7.1.2 Epidemiology ................................................................................................................................. 238
7.1.3 Characterization of C9orf72 phenotype .................................................................................... 239

7.2 Using information generated to direct future genetic studies............................................... 240

7.3 Conclusion.................................................................................................................................... 241
Table of Figures

Figure 1.1 ALS and ALS-FTD ........................................................................................................ 29
Figure 1.2 Classification of Neurodegenerative diseases ............................................................. 72
Figure 1.3 Factors leading to neuronal vulnerability .................................................................. 76
Figure 1.4 Disruption of axonal transport .................................................................................. 77
Figure 1.5 Mitochondrial dysfunction is implicated in ageing and degeneration .......................... 80
Figure 1.6 Proposed mechanisms for excitotoxicity ................................................................. 82
Figure 1.7 The neuroinflammatory cascade .............................................................................. 84
Figure 1.8 Common pathways leading to different clinical phenotypes ................................. 85
Figure 3.1 Respondents were given the option to say if they would consider the following kindreds to have FALS or not ........................................................................................................ 96
Figure 3.2 Average family structure (green=first-degree relative, red=second-degree relative, black=affected individual) ........................................................................................................ 99
Figure 3.3 The risk of having other family members affected by chance depending on kindred size where lifetime risk of ALS is 1/411 .......................................................................................... 100
Figure 3.4 Percentage of SOD1 positive and C9orf72 positive results from a cohort depending on the proportion of 2- and 3- affected family member kindreds included ....................................................................... 101
Figure 3.5 Forrest plot of the prospective and retrospective population based studies ................ 110
Figure 3.6 Geographic distribution in Europe of rates of FALS .................................................. 110
Figure 4.1 Demonstrating the increase in age at onset and diagnosis over time ......................... 124
Figure 4.2 Proportion diagnoses made in each age categories for all time periods ...................... 125
Figure 4.3 Proportion of cases diagnosed in each age category for SALS and FALS .................... 125
Figure 4.4 Corrected incidence rate plotted for each time period and age category .................. 126
Figure 4.5 Multivariate model of survival from diagnosis in different time periods (p=0.48) .......... 129
Figure 4.6 Attendance at the Beaumont ALS clinic was associated with a median survival time of 18 months from diagnosis of disease (95% CI 16.6-19.4), compared to a median survival of 10 months (95% CI 8.6-11.4) in those not attending the clinic (p<0.0001) ........................................................................................................ 130
Figure 4.7 Univariate comparison of survival in familial and sporadic ALS cases ....................... 131
Figure 4.8 An increased hazard rate of death is associated with older age at diagnosis of ALS ......... 132
Figure 4.9 Frequency of age at diagnosis in familial cases is normally distributed ...................... 133
Figure 4.10 Two subgroups identified from within the familial ALS cohort ................................................................. 134

Figure 4.11 Map with geo-coordinates of 1304 patients diagnosed from 1996-2011 in the Republic of Ireland. Map A, on the left, displays the geo-coordinates for sporadic patients (green dots), and Map B, on the right, familial patients (red dots) .................................................................................................................. 135

Figure 4.12 The list on the left demonstrates the rate of familial ALS compared to sporadic ALS per county in Ireland - Tipperary and Kilkenny are over represented; On the right there is a map of familial ALS cases in Ireland with a grey circle superimposed on counties Tipperary and Kilkenny (data from Irish ALS Registry 1996-2011) ........................................................................................................................................ 136

Figure 4.13 Corrected incidence per age category for Ireland compared to Northern Ireland, 2005-2010 ........................................................................................................................................ 139

Figure 4.14 Corrected incidence for each age category split by sex and country .......................................................... 139

Figure 4.15 Cumulative survival for patients from Ireland compared to Northern Ireland (p=0.0001).
Legend: Ireland (blue line), Northern Ireland (green line) ................................................................................................ 140

Figure 4.16 Figure A shows proportional age at onset of ALS, and figure B shows proportional age at diagnosis of ALS ........................................................................................................................................ 142

Figure 4.17 Scatterplot with regression line demonstrating mean age at onset versus life expectancy in all prospective and retrospective population based studies ........................................................................ 143

Figure 5.1 Flow chart showing the composition of the cohort of 191 cases recruited from the Irish ALS Register. ........................................................................................................................................ 151

Figure 5.2 Reverse-prime PCR results in multiple repeats on capillary electrophoresis. A positive result has 24 or more repeats in a decaying pattern ........................................................................................................................................ 154

Figure 5.3 Frequency of repeat number in patients and controls tested ........................................................................ 154

Figure 5.4 Repeat number in 396 patients who did not carry an expanded repeat .................................................................................................................. 155

Figure 5.5 Kaplan survival probabilities for patients with Amyotrophic Lateral Sclerosis (ALS) stratified for the presence of the repeat expansion ........................................................................................................................................ 158

Figure 5.6 Kaplan-Meyer survival curves demonstrating significantly longer survival in the familial cases of ALS without known genes compared to familial cases carrying the C9orf72 repeat expansion (p=0.015) ........................................................................................................................................ 159

Figure 5.7 Neuropsychological test results from ALS population-based cohort (n=186) ........................................ 160

Figure 5.8 VBM Analysis: Clusters of statistically significant grey matter atrophy in a cohort of ten ALS patients with the C9orf72 repeat expansion compared with thirty matched ALS patients without the
repeat expansion................................................................. 165
Figure 5.9 Screening algorithm (figures shown are actual numbers from our population based incident cohort).................................................................................. 166
Figure 5.10 Comparison between the subgroups identified from familial cases identified in the epidemiology chapter (figure A), and C9orf72 positive patients......................................................... 170
Figure 6.1 Section from the pilot family history questionnaire.................................................................................. 177
Figure 6.2 Family aggregation study logo: Family tree with fingerprint leaves and DNA tree trunk........ 178
Figure 6.3 Online 1911/1901 census (www.census.nationalarchives.ie) .......................................................... 183
Figure 6.4 Recruitment for the Family Aggregation Study.................................................................................. 187
Figure 6.5 A. Proportion of cases in each FALS definition category; B. Number of family members affected in each kindred.................................................................................. 204
Figure 6.6 Cumulative hazard stratified into groups based on repeat expansion status demonstrated in the figure. The table gives the hazard rates for each group compared to the hazard rate for relatives of controls (HR 1.0)................................................................................................. 205
Figure 6.7 Comparing penetrance estimates for each kindred using two different methods ....................... 206
Figure 6.8 Informative families without any known genes identified from family aggregation study (arrow denotes proband)................................................................................................. 208
Figure 6.9 Family history for candidate for exome sequencing (Arrow denotes proband, ALS patients with DNA were selected for Exome sequencing)................................................................................................. 209
Figure 6.10 Histogram demonstrating the frequency of dementia cases in families of cases compared to controls.................................................................................. 211
Figure 6.11 Hazard ratio of developing ALS in relatives of ALS patients with normal and abnormal cognition compared to controls.................................................................................. 215
Figure 6.12 Age at onset of dementia in relatives of patients is younger compared to relatives of controls.................................................................................. 216
Figure 6.13 A. Table demonstrating the frequency of offspring from cases and controls. B, C Histograms depicting the percentage frequency of offspring between cases and controls.................................................................................. 217
Figure 6.14 Depicts the percentage of time the frequency of offspring appears in cases and controls ...... 218
Table of Tables

Table 1.1 Factors that confound the ascertainment of an accurate family history ........................................ 36
Table 1.2 Genes associated with ALS (URL: http://alsod.iop.kcl.ac.uk/) ......................................................... 41
Table 1.3 Amount of genetic information shared depending on relatedness .................................................. 49
Table 1.4 Family aggregation studies of ALS ................................................................................................. 57
Table 1.5 Common clinical features of neurodegenerative diseases ............................................................ 71
Table 1.6 Neurologic diseases associated with aberrant protein structure .................................................... 73
Table 1.7 Neurologic diseases and selectively vulnerable cells ...................................................................... 77
Table 3.1 Questionnaire response breakdown by country of origin .............................................................. 92
Table 3.2 Breakdown of responses ................................................................................................................. 94
Table 3.3 Proposed criteria for Familial ALS ................................................................................................. 102
Table 3.4 Studies included in meta-analysis ................................................................................................. 108
Table 3.5 Meta-analysis results: Subgroup analysis and pooled analysis ....................................................... 109
Table 4.1 Number of cases diagnosed in each four-year period. Proportion of sporadic (SALS) and familial (FALS) cases reported in each period .......................................................................................... 121
Table 4.2 Sex and site of onset broken down by sex and time period ............................................................... 122
Table 4.3 The histogram depicts the frequency of age of onset in years (x-axis) for all cases (y-axis) diagnosed between 1996-2011. Table demonstrates the age at onset and diagnosis for the period of diagnosis, sex, and family history. Standard deviation is reported in brackets ........................................ 123
Table 4.4 Incidence rates of ALS for different time cohorts ........................................................................ 126
Table 4.5 Comparison of ALS prevalence .................................................................................................... 127
Table 4.6 Use of interventions in ALS over the four time periods, and attendance at the specialist ALS clinic in Beaumont Hospital ................................................................................................................. 127
Table 4.7 The frequency of the top-ten surnames on the Irish ALS Register compared to the frequency of the top-ten Irish surnames ........................................................................................................ 137
Table 4.8 Demographic features of ALS patients from Ireland compared to Northern Ireland, 2005-2010 .................................................................................................................................................. 138
Table 4.9 Comparison of prevalence between Ireland and Northern Ireland .................................................. 140
Table 5.1 Demographic information for ALS patients from the population based cohort (n=191) with the C9orf72 repeat expansion compared to patients without the repeat expansion ........................................... 156
Table 5.2 Difference in tests of executive function between repeat-positive and repeat-negative ALS patients

Table 5.3 Demographic information for ALS-FTD patients with the repeat expansion compared to ALS-FTD patients without the repeat expansion from the population-based cohort (n=30)

Table 5.4 Differences in behavioural scores changes on FRSBE test between repeat-positive and repeat-negative ALS patients. Higher scores indicate more behavioural impairment

Table 5.5 Demographic information for ALS patients with the repeat expansion compared to ALS patient without the repeat expansion who underwent 3-T MR imaging

Table 6.1 Baseline characteristics of the representative sample contacted (n=219) compared to the sample not contacted (n=147)

Table 6.2 Demographic details of those patients who returned their family history questionnaire versus those who did not return a questionnaire or declined to participate in the study

Table 6.3 Comparison of family histories collected between cases and controls

Table 6.4 Breakdown of relative clarification for relatives of cases and controls

Table 6.5 Comparison of mean ages for different categories of relative between cases and controls

Table 6.6 Cause of death by general category

Table 6.7 Compares the numbers of death certificates which match the reported case of death (Yes), those that do not match (No), and those that add a cause of death that was previously unavailable (No information available [NIA] so cert added information), and those certificates omitting a reported neurological or psychiatric cause of death. $X^2=9.0 \ p=0.032$

Table 6.8 Originally reported a family history of ALS

Table 6.9 Cases of familial ALS discovered through the family aggregation study

Table 6.10 Comparison of relatives, of all cases and all controls, demonstrates a significant relative risk of schizophrenia and suicide in relatives of all ALS patients compared to relatives of controls

Table 6.11 Comparison of relatives of (A) C9 positive cases with controls, and (B) C9 negative cases compared with controls in a Cox-regression proportional model

Table 7.1 Proposed criteria for Familial ALS
Appendices

Appendix A Questionnaire for consensus of definition study ........................................... 252
Appendix B Original questionnaire for family aggregation study ........................................ 257
Appendix C Revised questionnaire for family aggregation study ......................................... 278
Appendix D Specific causes of death ...................................................................................... 291
Appendix E Published articles arising from this thesis ......................................................... 295
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>Amyotrophic Lateral Sclerosis</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer Disease</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>C9</td>
<td>Chromosome nine</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FALS</td>
<td>Familial Amyotrophic Lateral Sclerosis</td>
</tr>
<tr>
<td>FrSBe</td>
<td>Frontal systems behaviour scale</td>
</tr>
<tr>
<td>FTD</td>
<td>Frontotemporal dementia</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome wide association study</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington Disease</td>
</tr>
<tr>
<td>IBMPFD</td>
<td>Inclusion body myositis with Paget’s disease and frontal dementia</td>
</tr>
<tr>
<td>MND</td>
<td>Motor Neuron Disease</td>
</tr>
<tr>
<td>NIV</td>
<td>Non-invasive ventilation</td>
</tr>
<tr>
<td>OMIM*</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson Disease</td>
</tr>
<tr>
<td>PNFA</td>
<td>Progressive non-fluent aphasia</td>
</tr>
<tr>
<td>SALS</td>
<td>Sporadic Amyotrophic Lateral Sclerosis</td>
</tr>
<tr>
<td>SNPs</td>
<td>single nucleotide polymorphisms</td>
</tr>
</tbody>
</table>
Chapter 1  Introduction to Amyotrophic Lateral Sclerosis

1.1 Clinical presentation

Motor Neuron Disease (MND) is a collective term given to disorders of the motor neuron. Amyotrophic Lateral Sclerosis (ALS) is the most common type, and is an incurable, relentlessly disabling, neurodegenerative disease, which causes death in half of affected people within three years of onset[1]. Almost ninety per cent of people are dead within five years of symptom onset, giving ALS a worse prognosis than many cancers[2].

1.1.1 Motor symptoms

Two presentation types predominate in ALS; i) bulbar onset in forty percent of patients, and ii) spinal onset in just under two-thirds of patients. Respiratory onset is seen in less than five per cent of patients, and this form of ALS leads to death more rapidly than bulbar and spinal onset, due to diaphragmatic weakness and failure of the intrinsic muscles of respiration. El Escorial diagnostic criteria classify the diagnosis as definite, probable, possible or suspected depending on the number of regions involved, and on the presence of upper and lower motor neuron signs[3, 4].

The clinical presentation of ALS is variable and a number of mimic syndromes must be excluded before a diagnosis is made[5]. Good prognostic indicators include a long period between symptom onset and diagnosis, spinal disease[6], and the absence of cognitive impairment[7].

1.1.2 Cognition in ALS patients

For many years it was believed that patients with ALS developed motor signs only with absence of cognitive impairment, but recent population-based incident studies demonstrate that 15% of patients with ALS develop frank frontotemporal dementia (FTD), and a further 35% develop some degree of cognitive impairment[8], reflecting a multisystem neurodegenerative disease.
Cognitive impairment in ALS patients can occur in a number of domains including frank frontotemporal dementia resulting in marked changes in personality with executive dysfunction (FTD), executive impairment which can present as a difficulty planning and carrying out daily tasks, and non-executive cognitive impairment (NECI) which can present with memory difficulties[7].

A combination of clinical, neuroimaging, and neuropathologic data suggest that ALS and FTD may form part of a disease continuum, with pure ALS at one extreme and pure FTD at the other[9]. In patients with pure FTD and no evidence of ALS, presenting symptoms are comprised of marked behavioural change (bvFTD) in 60%, problems with semantic language (SD) in 20%, and progressive non-fluent aphasia (PNFA) in 20% of cases[10]. However, in ALS patients who have coexistent features of FTD, the overwhelming majority develops the behavioural variant of FTD, rather than the semantic or aphasic form, indicating that some common final pathway may drive the syndrome[8]. Recent research by Elamin and colleagues demonstrates that in a longitudinal study of cognition in ALS patients, that those without cognitive impairment on initial assessment are unlikely to develop cognitive impairment as the disease progresses, while patients with some abnormality on initial testing have worsening cognition over the course of their disease[11]. From this one could draw the conclusion that rather than being a spectrum, with ALS on one end and FTD on the other end, that the presentation of ALS is clinically heterogeneous, and can be divided into two broad categories: ALS patients with 'pure sporadic ALS' with no evidence of cognitive impairment, and ALS patients with a predominance of executive cognitive impairment and behavioural change (see figure 1.1).
Cognitive impairment is a poor prognostic indicator in patients with ALS[7], therefore in prevalent patients the frequency of cognitive impairment is lower as impaired patients tend to die more quickly.

1.1.3 Treatment options in ALS

At present, riluzole is the only disease-modifying agent available to treat ALS. Riluzole slows progression of symptoms within the first 18 months and increases survival by three months in treated populations[12].

Research into disease-modifying interventions such as pharmaceuticals, stem cell therapy, and gene therapy, are underway[13]. A number of negative phase three clinical trials have been carried out, including trials of Lithium[14], and Ceftriaxone[15]. D-Pramipexole is currently undergoing phase a large multi-centre stage three clinical trial and initial results are expected in early 2013[16].

Interventions such as gastrostomy[17] and non-invasive ventilation (NIV) are beneficial in supportive management and there is evidence that these interventions may prolong survival in suitable candidates. Studies of the benefit of gastrostomy in ALS have reported mixed results in terms of survival[17,18], though patients with a poor clinical course, prominent bulbar symptoms, and marked weight loss require parenteral nutrition, and are thus more likely to be recruited into these studies. One small study demonstrated a survival benefit for NIV[19]. Despite these interventions, survival in ALS remains poor.
1.2 Epidemiology of ALS

1.2.1 Incidence of ALS

The incidence of ALS is relatively uniform across Europe at around 2.1 cases per hundred thousand per year. A systematic review of incidence of ALS across all ethnicities, demonstrated that lower standardised incidence rates were observed in Asian populations[20]. Recently a study reported that the incidence was lower in admixed populations such as Cuba[20]. This may reflect greater genetic heterogeneity among admixed populations, which results in lower disease risk among specific population subgroups.

An increased incidence has been reported in Guam, and on the Kii peninsula of Japan[21].

1.2.2 Gender

The reported ratio of males-to-females with the disease varies from 3:2 to almost 1:1, and some researchers have proposed that this is due to an ascertainment bias among registries[22]. ALS occurs about two years younger in men than in women. Reasons for this have not been clarified and in the past have been attributed to gender-based environmental exposure, including trauma and physical activity[23], but a large epidemiological study carried out in a Dutch ALS population did not show any association between physical activity and the risk of developing ALS[24]. It has also been suggested that the female hormones are protective[25]. A recent paper looking at endogenous female reproductive hormones and the risk of ALS demonstrated that a longer reproductive period was associated with longer survival (HR 0.96; p=0.01). The authors postulated that female reproductive hormones may have a neuro-protective effect[26].

1.2.3 Age at disease onset

Age of disease onset in ALS varies across populations and across historical cohorts. The mean age of onset of ALS in some European populations appears to be increasing although the adjusted incidence rates and frequency of disease in the very old do not appear to have changed. The age related incidence increases up to the age of 70 and then appears to decline afterwards[23]. This may reflect ascertainment difficulties at extreme age, or it may reflect
the true epidemiology of the disease. A lower age of onset is reported in population isolates where incidence rates are high, which may reflect a higher burden of genetic susceptibility[21]. Familial ALS (FALS) is also generally associated with a younger age of onset[27]. This has been attributed to a higher genetic burden in familial cases, causing earlier disease onset. Recently age of symptom onset has been linked to a locus on chromosome one[28].

A recent paper by Turner and colleagues[29] reports that more than half of all cases of ALS in a historical cohort (1850-1940) had disease onset before the age of 45 years compared to less than ten per cent of cases in modern times. The authors concluded that the higher proportion of younger patients in the historical cohort was unlikely to be due to poor life expectancy at the time of the study, however argument to the contrary was limited. One could argue that the decreased age at onset, discussed by Turner and colleagues, was a reflection of lower life expectancy. In developed countries, overall life expectancy is primarily dependent on a combination of genetic determinants that increase risk for certain life-shortening conditions (e.g. hypertension, lipid profile); and modifiable factors including diet, smoking, quality of life, and medical service provision. As ones life expectancy increases so may the biological reserve afforded by this increase, it is possible that patients may develop ALS at an older chronological age. Little work has been done looking at the differences in age at onset of ALS between regions and between different time periods, and to the relationship to life expectancy.

1.2.4 Survival

ALS is a neurodegenerative condition, which significantly decreases life expectancy. Median survival in western countries three to five years from symptoms onset[30]. Very few papers have demonstrated a significant survival benefit in recent years, with the majority of papers reporting no survival benefit, even after the introduction of gastrostomy and NIV [31]. Attendance at a multi-disciplinary clinic has been associated with a longer survival[32]. The type of survival analysis used is important. Most traditional survival studies in ALS estimate survival using the Kaplan-Meier method and Cox-proportional hazard methods,
which can co-vary for factors such as age at onset, sex, site of onset etc. Survival analyses are complex, and time period effects, which affect the whole population over a defined period, must be considered. A recent French paper[33] reported an increased survival in the most recent time cohort in a clinical based cohort, using a Bayesian technique for analysis which involved modeling of continuous predictors (splines) to assess trends in survival for different variables[34].

A recent study demonstrated that a prognostic score could be generated using six prognostic indicators which accurately predicted survival in 64% of cases [35].

1.2.5 Phenotype and genotype correlation

Almost half of all known ALS genes, FUS, TARDBP, OPTN, and C9orf72, have been discovered in the past four years[36]. Clinical registers with associated DNA banks can be extremely useful in the description of the clinical phenotype associated with these new ALS genes[37-39].

A recent paper described the disease phenotype in ALS patients carrying a mutation in TARDBP[39]. The data was collated by screening over 1,000 sporadic and familial French ALS cases from a register with associated DNA bank. Similar studies have described the ALS phenotype associated with the FUS gene[38]. A series of papers have been written describing the phenotype associated with C9orf72[40, 41]. A wide spectrum of phenotypes may exist for a single gene.

1.2.6 Benefit of prospective population-based ALS Registers

A number of prospective population-based ALS Registers are now in operation including those in Ireland, Northern Ireland, Scotland, Southern England, The Netherlands, France, the USA, Uruguay, and Italy.

Modern epidemiological studies have moved away from reports based on case series from a clinic population, which were often biased with younger ALS patients, more atypical features, and a higher proportion of familial disease. Population-based registers aim to enroll all ALS patients diagnosed in a defined region. This allows an accurate estimate of incident rates; all
patients diagnosed in a particular region in a 1-year period. This is important because prevalent patients tend are often those with longer survival, less cognitive impairment[8], and tend to be less representative of the ALS population as a whole[42].

The maintenance of a register requires multiple sources of ascertainment, accurate verification of the ALS diagnoses using El Escorial criteria, and ongoing follow-up to ensure accurate survival analysis. Registers include all patients and therefore avoid the bias of including one particular subgroup of patient over another[43]. This ensures that the natural history of the disease is accurately recorded, allowing accurate phenotype-genotype correlations, as described above.

Capture-recapture methods are employed to estimate the numbers of unobserved patients, and are calculated using the following equation[44], which calculates the total expected number of patients:

\[ N = \frac{(M + 1)(n + 1)}{(m + 1)} - 1 \]

*Equation 1 Capture-recapture estimate where M is the number of unique cases identified in the first source of capture, n is the number of unique cases identified in the second independent source, and m is the number of cases identified by both sources.*
1.3 Familial ALS

In the majority of cases, ALS is sporadic but in a small percentage of cases another family member may be affected. 'Familial ALS' or 'FALS' is the term given to the co-occurrence of more than one case of ALS within a family. The first description of familial aggregation of ALS was reported in 1850 by Aran, who described the case of a 45-year-old sailor, and his two uncles, who had a form of motor neuron disease. In 1880, Sir William Osler described the first multi-generational ALS pedigree, and many years later, this family played an integral part in the discovery of the SOD1 gene[45]. In 1955, Mulder and Kurland were the first to report a case series of ALS patients with affected family members and suggested that the rate of familial ALS was 10% of all ALS cases [46]. Since then numerous papers outlining familial ALS have been published. No differing clinical features have been described between sporadic and familial cases of ALS, however as discussed above there are epidemiological differences between age of symptom onset in the two groups.

1.3.1 Defining familial ALS

Despite frequent use of the term familial ALS among ALS neurologists, patients, and researchers, no universal definition has been described in the literature. Such a definition would clarify how many relatives need be affected, and what the degree of relatedness should be. Does an ALS patient with two affected siblings have the same type of familial disease as an ALS patient with only one affected distant cousin? Could chance co-occurrence of two sporadic cases within a family account for apparent familial ALS?

In a systematic review of the literature (described in section 3.2), only two articles reporting results on familial ALS provide a definition, and the two definitions differ[47]. This highlights the lack of consensus that exists between clinicians and researchers when using the term familial ALS. A paper by Valdmanis demonstrated that over half of familial ALS cases only had two affected family members[48]. This paper also highlights the lack of controlled prospective studies to determine the risk to other family members of developing ALS.
Absence of a universally accepted definition for FALS leads to uncertainty when including 'familial cases' in genetic studies, when discussing genetic counseling with family members, when comparing epidemiological studies between countries, and when comparing rates of genetic causes of FALS between research groups.

1.3.2 Rate of familial ALS

In 1955, Kurland and colleagues[46] published a case series of 58 patients with ALS in which 10% reported a family history of ALS. Though subsequent reviews have reported varying rates of FALS, a review of the literature finds that all of these papers ultimately reference the Kurland paper, and the figure of 10% remains a widely accepted population frequency of FALS[49-53].

A rate for familial disease calculated from a case series is not a satisfactory way to calculate the rate, as there is inherent bias in referral to single centres. Epidemiological studies have shown that specialist centres tend to see younger patients, and a higher number of cases. The use of a population-based register is the most accurate way of reporting the rate of familial ALS. There are, nonetheless, potential difficulties with the reporting of familial disease to registers (table 1.1). Once reported to a population-based register the ALS status in the reported relative may or may not be verified. The patient may also be unaware of the existence of other affected members or may be the first family member to be affected. In these cases the illness will be recorded as sporadic. It is necessary for registers to regularly update the family history status of patients.
False negative reporting of ALS due to:
- Lack of information on older generations
- The patient being the first in their family to develop ALS and not informing the centre when a subsequent relative is diagnosed
- Misdiagnosis of a relative with an incorrect condition
- Relatives dying earlier of other causes
- Low gene penetrance and small family size causing disease to appear sporadic
- Denial

False positive reporting of ALS due to:
- Patients overcalling the diagnosis of ALS in relatives
- Over reporting of ALS on death certificates

| Table 1.1 Factors that confound the ascertainment of an accurate family history |

Reported rates of FALS from population-based registers in the literature range from 1.5% to 11.6% [46, 50, 54-108]. A systematic review and meta-analysis is necessary to estimate the reported rate of familial ALS among epidemiological studies.

Given the factors listed above that may confound accurate reporting of FALS, the reported rate may differ quite substantially from the true rate of FALS. In order to report the true rate of FALS one would need to use a population-based register looking at family history in ALS patients over a defined period of time, with the aim of addressing as many of the factors associated with inaccurate rates of FALS as possible. No detailed genealogical studies of families of people with ALS designed to establish the true rate of FALS have ever been carried out.

1.3.3 Risk of other family members developing ALS

A common question voiced by relatives of patients with ALS in the neurology clinic is “What is my chance of developing ALS in the future?” Answering this question can be challenging, and the degree of risk depends on whether the index case has other affected family members (FALS), or appears to have sporadic disease. The risk to other family members is likely to vary depending on disease aetiology; in patients with sporadic ALS where there is no history of another affected family member, and the development of disease may be due to a combination of genetic susceptibility and environmental factors, the risk to other family
members is likely to be low, but in ALS patients with a monogenic cause for ALS, such as a C9orf72 repeat expansion, the risk to other family members may be much greater. The risk of two people in the same family having ALS by chance rather than because of heritable factors is not discussed in the literature.

A number of different methodologies have been used in studies to calculate heritability and inheritance risk of ALS in other family members:

**Twin studies:** Heritability is a measurement of the degree to which genes influence clustering within families and estimates are made through the use of twin-studies. To date the largest twin-study meta-analysis estimating heritability in ALS has been carried out by Al-Chalabi and colleagues[109]. This study estimates a heritability of ALS of 61%, meaning that genes accounts for almost two-thirds of the occurrence of ALS. If the heritability of a condition is high, then genetic interrogation of affected families may aid in the identification of new genes.

**Parent-child trio method:** Wingo and colleagues[110] used an ALS Register to determine the concordance of ALS occurrence among parents and offspring with ALS. Only 2.2% of patients with ALS (24) had an affected parent and concordance was noted to be least among fathers of affected women (0.5%). The authors reported that the lifetime risk of developing ALS was 1.1% in first-degree relatives of affected individuals. Given that the lifetime risk for ALS is estimated to be 1 in 350 in men and 1/472 in women (average 1/411) in the general population [95, 96], this result demonstrates just a 3.5- to 4-fold increase in the risk of ALS among the offspring of an affected parent.

**Use of multi-generational register:** Fang and colleagues[50] used a multi-generational register in Sweden to assess the relative risk of ALS in the first-degree relatives of affected individuals. There was comprehensive case ascertainment of 9,457 Swedish ALS patients using inpatient registration and death certification, but only 1,909 siblings were identified for analysis. This equates to just one sibling for every three patients with ALS. The authors acknowledge this shortcoming by stating that they could identify siblings for only 20% of the ALS cases who were born since 1932. Nine of the 1,909 siblings identified subsequently
developed ALS. The authors calculate a 17-fold increased risk for the development of ALS among the siblings of those affected. Since diagnoses were identified only through inpatient records or death certification, it is likely that more siblings with ALS were picked up than unaffected siblings, as they would be more likely to have been admitted to hospital or to have died. Of 6,671 ALS cases diagnosed between 1961 and 2005, only 46 relatives with ALS were identified, which gives a rate of familial ALS of only 0.7% in Sweden among siblings and children. A purely epidemiological study such as this, with incomplete sibling identification, and without consideration for disease phenotype among proband-sibling pairs may misrepresent the relative risk among siblings. To identify the rate of familial ALS and familial aggregation, a study needs to be conducted in a region where a prospective population-based ALS Register is in place, where DNA is banked, and where family history information can be collected on all parents, siblings, and children of patients with ALS, as well as second-degree relatives such as aunts, uncles, and nieces/nephews.

**Use of a longitudinal ALS Register:** Al Chalabi and colleagues looked at the development of ALS in relatives of sporadic ALS patients presenting to a specialist clinic without a family history at the time of diagnosis. Over sixteen years of follow-up just under 1% (26/3,167) of relatives subsequently developed ALS, and the authors calculated that the risk of first-degree relatives remaining unaffected by the age of 85 years dropped only marginally to 97.6% from 99.7% in the general population [111].

**Family aggregation studies:** The primary role of aggregation studies is to identify conditions that cluster in families by collecting detailed family histories from a representative proportion of sporadic and familial patients with the condition in question, and comparing the incidence in the family histories of matched controls. A number of family aggregation studies have been carried out looking at aggregation in families of patients with ALS and the methodology and results of these are discussed in detail in section 1.7. The largest of these family aggregation studies to date was published by Huisman and colleagues[112] in 2011 and reports a risk ratio of ALS among family members of patients with ALS compared to controls of 2.4 (95% CI 1.7–3.6).
Studies investigating the development of ALS among relatives of patients have shown a modest increase in lifetime risk. To date no study estimating the risk of developing ALS in family members of ALS patients have stratified for the presence or absence of specific genes. Such a study would likely identify marked differences between the risks of developing ALS in family members of patients with a monogenetic cause for ALS compared to those ALS patients with apparently sporadic disease.
1.4 Genetics in ALS

1.4.1 Single-gene causes of ALS

Inheritance is usually dominant with age-dependant penetrance, although recessive disease has been described in juvenile onset ALS and also in the SOD1 gene D90A mutation [45]. SOD1, the first gene to be associated with ALS, was described in 1993. The frequency of SOD1 mutations has been estimated to account for 10-20% of all FALS cases [52]. This estimate is not population-based and most studies preferentially recruit patients with familial ALS[54, 55]. To date, only one population-based estimate of SOD1 frequency has been carried out, reporting a rate of 13.6%[113]. Other genes known to be associated with FALS include SOD1, TARDP, FUS, ANG and the C9orf72 repeat expansion [36, 55]. Genes associated with ALS are presented in table 1.1. An online database (ALSOD) records all genes associated with ALS [114]. Genes may also be population specific depending on founder effects; the A4V mutation of SOD1 is extremely common in the US but much less frequent among Europeans [115]. C9orf72 has now been described in most populations, but there is evidence to suggest that a single founder may exist[116].
### Table 1.2 Genes associated with ALS (URL: http://alsod.iop.kcl.ac.uk/)

<table>
<thead>
<tr>
<th>ALS gene</th>
<th>Locus</th>
<th>Gene</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS 1</td>
<td>21q22.1</td>
<td>SOD</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 2</td>
<td>2q33.1</td>
<td>Alsn</td>
<td>Recessive (juvenile)</td>
</tr>
<tr>
<td>ALS 3</td>
<td>18q21</td>
<td>SETX</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 4</td>
<td>9q34</td>
<td></td>
<td>Dominant (juvenile)</td>
</tr>
<tr>
<td>ALS 5</td>
<td>15q15-21.1</td>
<td></td>
<td>Recessive</td>
</tr>
<tr>
<td>ALS 6</td>
<td>16q12</td>
<td>FUS</td>
<td>Dominant/Recessive</td>
</tr>
<tr>
<td>ALS 7</td>
<td>20p13</td>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 8</td>
<td>20q13.3</td>
<td>VAPB</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 9</td>
<td>14q11</td>
<td>ANG</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 10</td>
<td>1p36.22</td>
<td>TARDP</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 11</td>
<td>6q21</td>
<td>FIG4</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 12</td>
<td>10p13</td>
<td>OPTN</td>
<td>Dominant/Recessive</td>
</tr>
<tr>
<td>ALS 14</td>
<td>9p13.3</td>
<td>VCP</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 15</td>
<td>Xp11.21</td>
<td>UBQLN1</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALSFTD1</td>
<td>9q21-22</td>
<td>C9orf72</td>
<td>Dominant</td>
</tr>
<tr>
<td>ALSFTD2</td>
<td>9p13.3-21.3</td>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>ALS 18</td>
<td>17q13.2</td>
<td>PFN1</td>
<td>Dominant</td>
</tr>
</tbody>
</table>

#### 1.4.2 Oligogenic theory

Recently a paper published by Blitterswijk and colleagues [117] demonstrated the existence of two or more different pathogenic ALS genes in five of 97 familial ALS cases tested for known genes associated with ALS. The study was informed by the observation that while a dominant pattern of inheritance was frequently seen in familial ALS, the penetrance was often incomplete. They hypothesized that this was because the inheritance of ALS in some familial cases may be due to the presence of more than one gene associated with ALS (oligogenic theory). The authors reported that the presence of multiple mutations in familial cases was more than would be expected by chance, and that this may in part explain why there may be so much phenotypic variation among ALS patients within the same families. This finding has implications for the design of future genetic studies in ALS, as patients with known genes are often excluded from analysis. The oligogenic theory also makes genetic
counseling in ALS more difficult, and may in part explain why some members of the same family develop ALS while others develop FTD.

1.4.3 Complex genetics of Sporadic ALS

In sporadic cases of ALS, where there is no evidence for a single gene of large effect, it is believed that multiple susceptibility genes of smaller effect, possibly influenced by environmental factors, can lead to the development of sporadic disease. However, a single gene with low penetrance may appear to be sporadic disease [118].

A number of studies have identified susceptibility genes, including VEGF, PON, and HFE, although not all have been replicated[119]. Susceptibility genes in ALS were originally identified using candidate gene studies, and have been superseded by genome-wide association studies (GWAS), which allow the identification of susceptibility genes in a large cohort of affected patients compared to controls (methods described later in the chapter). If multiple susceptibility genes found to increase the risk of developing ALS then consideration should be given to protective genes that may decrease the risk, or which may confer relative longevity in certain ALS patients.

1.4.4 Methods of gene discovery in ALS

Genetic Epidemiology is a discipline that combines epidemiology, biostatistics, and human genetics to determine the genetic basis for disease[120]. Following descriptive epidemiology of the disease in question, family aggregation studies are carried out to demonstrate that the disease runs in families to a higher degree than would be expected by chance. Segregation analysis is then carried out to determine whether the inheritance pattern is compatible with one gene of major effect or polygenic inheritance, then efforts are made to refine the locus of the gene either by using linkage analysis and fine mapping techniques in large families with many affected members or more recently through the process of exome sequencing. Once a potentially pathogenic gene is identified the pathophysiology is described in detail, and finally the phenotypic expression is described [121].
The advance in technology, since the discovery of the SOD1 gene, has been rapid, and techniques for gene discovery in familial ALS cases have graduated from linkage analysis in families with multiple affected members, and more recently onto next generation sequencing, and exome sequencing techniques.

Attempts to find genes associated with sporadic ALS cases have been carried out with candidate gene analysis, and GWAS.

1.4.5 Linkage analysis

Linkage analysis is the original method of genetic analysis that first allowed identification of Mendelian genes of large effect size in affected individuals with ALS.

The premise for the method is simple: initially a large pedigree is selected where multiple family members over a number of generations are affected with a similar phenotype of disease. DNA must be available on a large number of affected and unaffected individuals in order to identify a small region on a chromosome where a putative gene may lie. The presence of certain markers in the DNA samples from each family member is identified to determine which alleles were present in the affected individuals and absent in those who were free from the disease phenotype. Using marker information in affected individual a LOD score, which is a statistical estimate of the likelihood of two markers in close proximity on a chromosome (and therefore having been inherited together as a unit), is calculated. The locus with the highest LOD score is most likely to contain the gene causing the phenotype.

In the past Sanger sequencing ('first generation sequencing') was performed on the region of interest to identify the gene. Compared to 'next generation sequencing', Sanger sequencing was relatively slow and costly, and as a result extended the time to gene discovery. High throughput methods produce thousands of sequences at once, and have essentially revolutionised the pace and cost of genetic discovery. Prior to this, researchers often spent many months to years working on novel gene discovery in large kindreds.

Upon discovery of a pathogenic gene using linkage analysis, control populations are screened for the presence of the gene to ensure that it is not a rare variant. If it is not present in a large number of control subjects, the disease population is tested for its presence.
Linkage analysis was useful in identifying single genes of large effect in multiple affected family members. Genome-wide association studies are useful in complex diseases which may involve many genes with small to moderate effect size. As linkage studies are only possible when DNA is available from multiple affected and unaffected members from a large kindred over a number of successive generations (especially when the disease in question occurs in middle- to older-age), traditional family linkage studies have been superseded in recent times by exome and whole genome sequencing studies.

1.4.5.1 ALS genes discovered through linkage analysis within a family

Although traditional linkage studies have been superseded in recent times by newer methods they have been very successful in the discovery of Mendelian genes. In fact, a number of ALS genes were discovered in this way:

*SOD1*: In 1880, Sir William Osler, described the case of the Farr family of Vermont. This family had a dominantly inherited form of ALS. Over a century later in 1993, genetic samples from descendants of this family were used in linkage studies which lead to the identification of the *SOD1* gene [122]. To date over 150 apparently pathogenic mutations in the *SOD1* gene have been reported, although not all mutations have been demonstrated to be pathogenic. This may be the reason for non-segregation of *SOD1* genes in families where multiple members have ALS [123].

*FUS* gene: The fused-in-sarcoma gene was discovered in 2009 to be associated with ALS using a large pedigree. *FUS* was first reported to be associated with liposarcoma in 1993 [124]. The authors of the linkage study which proved the association of *FUS* with ALS, had previously reported linkage to a 42 Mb region on chromosome 16, but it was not until a further two family members developed ALS that they could further refine the locus which contained over 400 genes. The authors took a candidate gene approach and sequenced 279 exomes from 32 genes. They were able to identify a 1561 C>T (R521C) mutation that segregated with all six affected family members. The authors estimated that the frequency of *FUS* was 4% in ALS cases [125, 126].
9p locus: In 2000, Hosier and colleagues [127], used linkage analysis in sixteen families to identify a 17cM locus in 9p21 that correlated with familial ALS-FTD. Other groups consistently replicated this linkage association[128]. These were the first studies to link ALS and FTD to the 9p region, a full description of the discovery of C9orf72 is included in section 1.5.

1.4.6 Genome-wide associations studies

The genome-wide association study (GWAS) is a technique used to identify genetic traits that may be associated with the risk of developing a particular disease. Instead of focusing on the discovery of single genes of large effect, GWAS compare the presence of many thousands of single nucleotide polymorphisms (SNPs) in a large number of affected individuals and unaffected controls to determine regions that may be associated with an increased risk of developing disease [129]. This method may lead to gene discovery in sporadic cases were the genetic aetiology may be complex, involving a number of susceptibility genes associated with developing the condition rather than one single gene. Dunckley and colleagues[130] were the first to report a genome-wide study in ALS. They identified 34 SNPs of interest but none were significant following Bonferroni correction.

GWAS techniques did identify a region of significance in the 9p21 locus [131-133], however this was discovered because of the inclusion of some familial cases of ALS, and apparently ‘sporadic’ ALS cases that did not disclose a family history of ALS. GWAS is very useful in identifying susceptibility genes for complex traits, however the risk attributed to many of these genes is very small.

1.4.6.1 ALS genes discovered through GWAS

9p21 locus: In 2009, van Es and colleagues [131] used the Genome Wide Association Study (GWAS) method to compare 2,323 individuals with sporadic ALS to 9,013 controls. They identified two single nucleotide polymorphisms (SNPs) that reached genome wide significance in the region, 9p21. The authors then replicated the findings in an independent cohort of 2,532 affected individuals and 5,940 controls. Two further large studies confirmed
these findings[132, 133]. For the next two years numerous attempts to sequence this area using next generation sequencing methods failed to identify a pathogenic mutation in the region. A full discussion of the discovery of C9orf72 is in section 1.5.

1.4.7 Candidate gene approach

The candidate gene approach is used when a locus of interest is identified through linkage analysis, GWAS, homozygosity mapping, or through pathway analysis[134]. In conditions where the disease appears to be inherited in an autosomal recessive pattern, rapid genotyping of single nucleotide polymorphisms (SNPs) across the genome in a small number of affected individuals can identify regions of homozygosity that segregate with the disease phenotype. This process requires very few affected individuals, and at present, takes a matter of days. The regions of homozygosity are then interrogated to identify candidate genes. When regions of interest are identified using the techniques outlined above, sequencing is carried out in order to identify a mutation.

1.4.7.1 ALS genes discovered through candidate gene studies

ANG gene: In 2006, Greenway and colleagues described the association of the Angiogenin gene (ANG) with ALS. They used a candidate gene approach based on the allelic association of ANG with the Apurinic Endonuclease gene (APEX) in the Irish population. They sequenced the coding region of ANG in 1,629 individuals with ALS and 1,264 controls. They identified seven missense mutations in 15 individuals, of whom four had familial ALS and 11 sporadic ALS [135, 136]. Mutations were subsequently discovered in a number of controls, and ANG is now established as a risk factor for ALS, conferring a substantial risk (odds ratio 9.2)[137].

TARDBP gene: The presence of ubiquinated cerebral inclusions is the pathological hallmark for ALS, tau-negative FTD, and numerous other brain disorders. TAR DNA binding protein (TDP-43) comprises most of the proteinaceous content of the ubiquinated inclusions in ALS and non-tau FTD. The authors chose TARDBP as a candidate gene due to the phenotypic overlap between ALS and FTD. They identified a mis-sense mutation in exon 6 of TARDBP in
affected individuals from a large ALS pedigree and then confirmed the presence of the mutation by carrying out linkage analysis within the large kindred\[138, 139\].

**OPTN gene:** In 2010, Maruyama and colleagues used homozygosity mapping to identify a small region in chromosome 10, with 17 candidate genes, in six Japanese individuals from consanguineous marriages. They sequenced the exons of the candidate genes and detected a deletion in exon 5 of the Optineurin gene (\textit{OPTN}) that segregated with affected members in families[140].

### 1.4.8 Exome Sequencing

The process of exome sequencing involves choosing a family where a number of members are affected by one particular disease and then exome sequencing one affected member with the aim of identifying a pathological gene[141]. Bioinformatic subtraction methods are then used to remove any known variants from the exome results of that individual. A small number of variants found to be present in the affected family member can then be screened for in the other family members. Ideally, one variant will segregate with all affected family members and be absent in unaffected family members.

Exome sequencing technology uses DNA-enrichment methods and large scale parallel nucleotide sequencing to identify and type exonic protein-coding regions. These protein-coding regions represent less than 2% of the human genome. When used in conjunction with reference databases containing known variants, such as dbSNP and 1000 Genomes which can be used to exclude non-pathogenic variants, exome sequencing allows identification of genetic mutations in affected individuals. Prior to exome sequencing, multiple affected family members were required to calculate LOD scores but new exome sequencing techniques can refine the search to a few base pairs and require DNA from fewer affected family members. This technology is effective at identifying missense, non-sense, splice site, and small insertion or deletion mutations in exonic regions but is unable to detect larger repeat mutations. Exome sequencing of one individual, depending on ethnicity, will typically yield around 20,000 variants. Data are then compared to reference genomic information and up to 19,300 of these variants may be accounted for by known variants and subsequently
If the proposed mode of inheritance is recessive this further reduces variant number 50-fold. At this stage exomic data from a distant relative may be added to further refine the search for a putative gene. The same region can be sequenced in another affected relative to see if both affected relatives share the same mutation. Finally, candidate disease variants can be interrogated using bioinformatic methods, and variants without any proteomic function or not involved in the disease pathway can be excluded.

Exome sequencing is only used in the discovery of mutation in the coding regions, and cannot sequence the 98% of the genome that is intronic, or identify structural variants. The current hybridization technique often fails to cover substantial parts of the exome (5-10%), which may result in missing pathogenic mutations [141].

### 1.4.8.1 Choosing candidates for exome sequencing

Candidates for exome sequencing projects must be carefully chosen as sequencing is expensive. Methods for choosing candidates include choosing relatives from within the same family, patients with an 'extreme phenotype', and a parent-child study if a child has a disease which is believed to be caused by a novel mutation.

**Family study:** Despite the fact that linkage techniques have been superceded by exome sequencing in kindreds, the importance of identifying large families with multiple affected members remains important for gene discovery. Unlike linkage studies, fewer affected and unaffected family members are required for exome sequencing projects and discoveries have been made using only two affected individuals. A small number of relatives (as few as two) who are ideally farthest apart genetically are then chosen and exome sequencing is carried out on their samples. It is best to choose individuals farthest apart genetically as they share the smallest amount of DNA (table 1.3). Bioinformatic analysis will identify regions shared by both individuals and cross-reference with online resources will allow the researcher to identify a region which may carry a pathogenic gene.
<table>
<thead>
<tr>
<th>Degree</th>
<th>% DNA shared</th>
<th>Number genes shared</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>50.0%</td>
<td>15,000</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>25.0%</td>
<td>7,500</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>12.5%</td>
<td>3,750</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>6.3%</td>
<td>1,875</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.1%</td>
<td>938</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.6%</td>
<td>469</td>
</tr>
<tr>
<td>7&lt;sup&gt;th&lt;/sup&gt;</td>
<td>0.8%</td>
<td>235</td>
</tr>
</tbody>
</table>

*Table 1.3 Amount of genetic information shared depending on relatedness*

Trio study: Exome sequencing is used to identify a novel mutation in the offspring of two parents. DNA must be available on both parents and the affected child.

Extreme phenotype study: Cases which display an 'extreme phenotype' are chosen on the assumption that cases sharing marked clinical features also share a pathogenic gene.

1.4.8.2 ALS genes discovered through exome sequencing projects

*Profilin* gene: Two large families were included in an exome sequencing study. Mutations within the *PFN1* gene were identified [143].

*SPG11* gene: Exome sequencing of two individuals within a kindred, where one had juvenile onset ALS and the other had spastic paraparesis, identified a mutation in the *SPG11* gene [144].

*VCP* gene: Exome sequencing was used to identify the segregation of *VCP* in family members affected with ALS in an autosomal dominant pattern [145].

1.4.9 Gene Penetrance

Penetrance is defined as the proportion of people carrying a gene who develop the phenotype related to the gene during the course of their lifetime. Segregation analysis determines the mode of inheritance of a gene or phenotype within a kindred.
Al-Chalabi and colleagues recently reported the finding that family size and gene penetrance directly contribute to observed inheritance patterns within a kindred, and that poorly penetrant genes in small families may appear to cause sporadic disease[118].

In the USA, 5% of the sporadic ALS population carry a mutation in the SOD1 gene. These cases may represent familial disease with low penetrance[146]. Alternatively, SOD1 mutations are reported without evidence that the mutation is truly pathological, which might explain why the SOD1 gene does not always segregate in affected family members. A large study recently demonstrated that clear segregation of the SOD1 gene and phenotype only occurred in 5/28 families[123]. Estimation of penetrance for specific genes can be useful in genetic counseling.
1.5 Discovery of the \textit{C9orf72} repeat expansion in ALS

In the late nineteen-seventies and early nineteen-eighties, single reports of ALS patients presenting with socially inappropriate and impulsive behaviour, and a general deterioration in the ability to perform routine daily tasks consistent with fronto-temporal dementia, were documented in the literature. Following this observation, aggregation of ALS, FTD or ALS-FTD in an autosomal dominant pattern was consistently reported in large pedigrees.

In 2000, Hosier and colleagues [127], used linkage analysis in sixteen affected families, where members had ALS, FTD or a combination of both ALS and FTD, to identify a 17cMb locus in 9p21 that correlated with familial ALS-FTD. Other groups consistently replicated this linkage association[128].

In 2009, van Es and colleagues [131] used the Genome Wide Association Study (GWAS) method to compare 2,323 individuals with sporadic ALS to 9,013 controls. They identified two single nucleotide polymorphisms (SNPs) that reached genome wide significance in the region, 9p21. The authors then replicated the findings in an independent cohort of 2,532 affected individuals and 5,940 controls. The finding of a significant association of ALS with 9p21 was replicated in a large GWAS study pooling data from several European countries to increase power [131-133].

For the next two years, despite numerous attempts to sequence this area using next generation sequencing, no pathogenic mutation was identified in the region. This is because the gene was a repeat expansion in an intronic region. In late September 2011, two independent research groups, one working in the field of ALS and the other in the field of FTD, simultaneously reported the presence of an expanded hexanucleotide repeat expansion in the non-coding intronic region 9p21 that segregated with ALS, FTD and ALS-FTD in affected families[147, 148]. The groups used two different methods to find the repeat expansion: one group used traditional segregation analysis and the second group used whole genome sequencing in the 9p21 region. This is a very significant finding as the repeat expansion may be associated with over half of all familial ALS cases.
A number of repeat disorders are associated with other neurological conditions such as Huntington's disease and Myotonic Dystrophy. Kennedy's syndrome is an X-linked repeat disorder that presents with signs and symptoms of spinal onset ALS but is associated with normal survival. These conditions can be associated with anticipation from generation to generation. There is also some evidence to suggest that fecundity may be impaired in people with repeat disorders: recently altered reproductive function has been described in females with myotonic dystrophy[149], and fertility in pre-mutation carriers (55-200 repeats) of the fragile-X gene is impaired due to premature ovarian failure and altered reproductive function may also precede ovarian failure by many years thus limiting offspring number even further[150].

Attempts are now underway to understand the clinical phenotype and pathophysiological mechanisms associated with this mutation. The discovery of the C9orf72 repeat expansion highlights the importance of gene discovery using the methodology of genetic epidemiology.
1.6 Genetic counseling in ALS

At present there is limited information available to the neurologist or clinical geneticist with regard to advising family members about their individual risk of ALS.

Genetic counseling is a specialty concerned with educating family members about the medical, psychological and familial implications of genetic diseases. The procedure involved in genetic counseling typically involves: i) the interpretation of family and medical histories to assess the chance of disease occurrence, ii) education about inheritance, testing, and management, and iii) counseling to promote informed choices and psychological adjustment to the risk or condition.

Predictive testing involves the investigation of asymptomatic individuals with a family history of a condition to determine whether or not they carry a pathogenic gene. A positive gene test is not necessarily diagnostic of the disease. Likelihood of the disease occurring in the future depends on the penetrance of the gene. The disease may be inevitable (Huntington’s Disease if more than 35 \(CAG\) repeats are present), likely (80% lifetime risk of cancer with \(BRCA1\)), or less likely if the penetrance is low. In single-gene disorders, which are preventable through screening (cancer) or through intervention (regular phlebotomy for haemachromatosis), the uptake for genetic testing is usually high. In single-gene disorders where the disease may be considered devastating, and no effective therapeutic intervention is available, the uptake is lower and should not be undertaken without adequate genetic counseling. Family members seeking a ‘genetic test’ may be looking for a test for a single-gene disorder such as Huntington’s disease (HD) where the outcome is either positive or negative, but may also present requesting a genetic test for ‘dementia’ because relatives, who may have died without ever having had a genetic test, developed dementia in later life. In conditions such as dementia where many genes, both known and unknown, as well as environmental factors, play a role, it is much more difficult to advise the patient requesting testing as there is often no definitive answer.
At present, genetic counseling in ALS is difficult – *SOD1* accounts for 20% of familial cases (2% of all ALS cases), but this rate is highly variable between different populations, penetrance is often incomplete and phenotypes are highly variable[151]. With the recent discovery of the *C9orf72* repeat expansion, which may account for half of all familial cases of ALS, genetic counseling is further complicated as the penetrance is unknown and the phenotype may be variable [40]. These factors make predictive counseling for relatives of people with ALS difficult.
1.7 Family aggregation studies in ALS

The identification of families with multiple affected family members is essential for the discovery of new genes. The observation that there was an over representation of ALS and FTD cases within families ultimately led to the discovery of \textit{C9orf72} repeat expansion. The aim of this section is to provide a detailed literature review and discussion of the concept of aggregation of disease within families, and to evaluate the methodology used in these studies, in order to create a well designed family aggregation study of ALS in an Irish population. Particular attention has been paid to studies where medical conditions other than ALS aggregate within a family where at least one member had ALS.

1.7.1 Introduction to family aggregation

'Family aggregation' refers to the occurrence of a trait in more members of a family than can be readily accounted for by chance. The primary role of aggregation studies is to identify conditions that cluster in families and identify causative genes. The discovery of new disease-causing genes and the elucidation of their function sheds light on the pathophysiology of disease and hopefully results in advances in therapeutics with an attendant reduction in mortality and morbidity. Family aggregation studies also provide vital information for genetic counseling.

Family aggregation of traits such as physical characteristics has been noted for many thousands of years. More recently scientists have observed the aggregation of diseases in families. When one thinks of aggregation of a trait within a family, there is a tendency to assume that the factor driving aggregation of the trait is genetic, but there are three major factors account for aggregation of traits within families: i) genetic factors; ii) environmental factors; and iii) sociological factors.

\textbf{Genetic factors:} Genetic factors account for the majority of diseases known to aggregate within families. Disease segregation within a family depends on the penetrance of the gene, the pattern of inheritance, and the size of the family. The first description of the genetic
factors associated with physical traits were seen in letters written by Gregor Mendel
describing his ‘Laws of inheritance’. At the time of writing (22/7/2012), 14,114 disease-
causing genes have been described in OMIM® (Online Mendelian Inheritance in Man)[152].

Environmental factors: Co-habiting families are exposed to common environmental factors
such as food and water sources, and similar pathogens. For example the formerly common
practice of insulating homes with asbestos was responsible for the co-occurrence of
mesothelioma among family members sharing a home.

Sociological factors: In the past, subsequent generations of a family would often share a
particular trade. The tendency for an occupation to ‘aggregate’ within a family can be
explained, in the main, by the societal practice of father passing his trade onto his son and so
on.

Traditionally family aggregation studies investigated the aggregation of only one disease
within a family (e.g Alzheimer disease), but more recently studies have been designed to
investigate the aggregation of two or more conditions within a kindred. Recently, a paediatrican
looking after children with Gaucher disease, made the observation that many of their grandparents
had Parkinson’s disease (PD). Neudorfer and colleagues [153, 154] first reported that Gaucher
disease tended to aggregate within families of people with PD. Mitsui and colleagues[155]
subsequently demonstrated that heterozygous carriage of a pathological variant of the gene which,
in the homozygous state, causes Gaucher disease, is a risk factor for the development of PD.
Recognition of two disorders co aggregating can aid in gene discovery.

1.7.2 Family aggregation studies in ALS

A Medline literature search was performed to identify all published studies reporting family
aggregation studies in ALS, from 1966 to April 2012.

Seven studies were identified which reported aggregation of medical conditions within ALS
kindreds. The methodology from these studies are presented in table 1.4. Most of the studies
focused on the aggregation of neurodegenerative disease among family members, although
one study did look at the aggregation of vascular disease among the relatives of ALS patients.
Study 1 - Deapen et al, 1986: Between 1977 and 1979, the authors carried out an epidemiological study, using the self-report questionnaire method, to look at possible risk factors for ALS in 518 patients and in an equal number of matched controls [156]. Patients were identified through the ALS society of America and represented both incident and prevalent cases. The primary aim of this study was to investigate the relationship between exposure to physical trauma and the risk of developing ALS. Obtaining family history data was a secondary aim. Respondents were asked about the presence of eleven neurological conditions in first-degree relatives only.

The authors reported that patients who responded to the questionnaire tended to have a younger age at diagnoses than those on the register who did not respond.

The only condition occurring more frequently in relatives of cases was PD, with an odds ratio of 2.7 (95% CI 1.1-7.6). An increased rate of Poliomyelitis was also seen among first-degree relatives but this did not reach statistical significance (OR 1.8, 95%CI 0.9-3.7). This study did not include dementia.

Study 2 - Armon et al, 1991: In 1991, Armon and colleagues reported a family aggregation study with 74 ALS cases and 201 matched controls[157]. The study investigated a large
number of potential risk factors including i) evidence for sporting prowess, ii) family history of neurodegeneration, iii) lead exposure, iv) rural versus urban dwelling, and v) evidence of trauma. The questionnaire administered to the patient did not differentiate between the specific neurodegenerative diseases among relatives, nor did they specify the degree of relatedness. There was no significant difference between the burden of neurodegenerative disease reported between the ALS cases and matched controls.

**Study 3 - Savettieri et al, 1991:** This small epidemiological study looked at numerous putative risk factors for ALS in 46 patients and 92 matched controls[158]. A standard interview was conducted with each participant. Patients were asked about 'neurological disease' among first-degree relatives but the paper does not specify how this was defined. To increase power the authors matched one patient to two controls and then carried out triplet analysis. Patients reported more neurological disease among first-degree relatives but this did not reach statistical significance (OR 2.2, 95%CI 0.4-11.0). Despite attempting to increase power with the use of triplet analysis, small numbers mean that power was insufficient to detect small differences between groups.

**Study 4 - Majoor-Krakauer et al, 1994:** Majoor-Krakauer and colleagues reported the first comprehensive aggregation study of dementia and Parkinson's disease (PD) in family members of patients with ALS[159]. They conducted a study of 151 ALS patients recruited from a single centre. Only ALS patients without dementia were recruited, although no formal cognitive testing was carried out. 140 controls were recruited from age-matched neurological inpatients without a diagnosis of dementia or PD. Participants completed a semi-structured interview inquiring about dementia and PD in first-degree relatives and grandparents (second-degree, although no other second degree relatives were included). Information on the presence or absence of dementia was only available on 32% of the grandparents of patients.

Attempts to verify the information were made by requesting autopsy reports and medical notes. Despite collecting data on 2,258 relatives, corroborating information was available only for 43 (2% of all relatives). Only 23 of those 43 records verified the diagnosis.
Survival analysis methods were used to control for differences in the years-at-risk of each disorder. This method is useful for studies looking at disorders with onset in later life, where some family members are too young to have expressed the disease phenotype and others have died of another cause before manifesting the disorder. Cumulative incidence estimates were then calculated to estimate the risk of manifesting the condition by a particular age to a relative of a patient with ALS compared to the relative of a control. Cox proportional hazards analysis was used to calculate the rate ratio for each disorder in relatives of ALS patients versus controls.

The reported rate of FALS among this cohort was 4.6%. The rate of dementia was twice (1.9) as high in relatives of ALS patients as it was in relatives of controls. Importantly, of the seven papers, this is the only paper to report confidence intervals for the relative risk of dementia in relatives of patients with ALS that are statistically significant (95% CI 1.1-3.2).

In the discussion the authors note that ALS is likely to be heterogeneous and that the shared genetic susceptibility for other neurodegenerative diseases is likely to only be found in a subset of families.

Study 5 - Cruz et al, 1999: This is a population-based case-control study looking at epidemiological risk factors in 174 ALS patients and 348 controls[62]. Controls were selected through a very complex recruitment system, which involved identifying 4,858 residential telephone numbers and then screening them to identify 262 suitable controls. A further 202 eligible controls were identified through a Medicare list.

Patients reported a family history of ALS in a first- or second-degree relative in 5% of cases. The study demonstrates that patients have a higher number of first- and second-degree relatives with ALS than controls (OR 3.3, 95% CI 1.1-9.9). Slightly more cases had a family history of AD and slightly more controls had a family history of PD but these results did not reach statistical significance. This may be explained by the fact that collection of information was limited to first-degree relatives only.

Study 6 - Fallis et al, 2009: This is a retrospective chart review, which reviewed the pedigrees of 197 ALS cases and 235 patients attending a general neurology clinic[160]. The
occurrence risk of ALS, PD, and dementia was calculated in first-degree relatives. The authors note that although the presence of neurodegenerative disease was increased among the relatives of patients with ALS, that it was not uniformly distributed across all families. The relative risk of neurodegenerative disease in family members of ALS patients compared to family members of controls was 4.0, although no significance level was given. The authors of this study postulate that there is likely to be a shared genetic susceptibility towards a neurodegenerative phenotype in certain families.

This is a retrospective study carried out in a clinic population in Ireland. The control cohort was drawn from attendees to a general neurology clinic that had presented with conditions such as migraine, headache, dizziness, back pain, and idiopathic epilepsy and patients and controls may not be matched appropriately. It is an important study as it was the first to point out that there was clustering of neurodegeneration within certain families.

**Study 7 - Huisman at al, 2011:** Huisman and colleagues report the largest family aggregation study performed to date [112]. This prospective population-based study of Dutch ALS patients used a self-report questionnaire to gather information on the presence of PD, dementia and cardiovascular disease in the relatives of 635 ALS patients and 1,616 matched healthy controls.

The cohort included all incident patients who were diagnosed between January 1st 2006 and December 31st 2009. They also included all prevalent patients, diagnosed before 2006 but who were alive on January 1st 2006. The proportion of responses from incident and prevalent cases was not reported. This is relevant as incident and prevalent ALS patients differ in that prevalent patients tend to survive longer and are less likely to be cognitively impaired.

The authors achieved a very high response rate from their questionnaires (87%) and they included data from 72% of patients eligible for inclusion in the study. The controls were matched by contacting the patients' family doctors and getting the names of three patients similar in age.
The authors defined the term FALS as follows 'Every patient who had a first-, second-, or third-degree family member with ALS was defined as having familial ALS (FALS). Of the patients with ALS, 6.4% reported a family history of ALS.

Questionnaires were self-reported, and although the researchers contacted the respondent upon return of the form, no further attempts to verify the information were made. No information was collected on aunts or uncles of the probands. Fifteen percent of patients with ALS have frank frontotemporal dementia, while a further 35% have some degree of cognitive impairment[8]. For this reason self-report may not be the best way to record family history. Patients with memory impairment may not remember to include an affected family member, while patients with executive dysfunction and may have difficulty completing a questionnaire.

Relative risk was calculated for relatives of cases compared with the relatives of controls. A linear mixed-effect model (maximum likelihood) using a binomial link function was used to account for the non-independence of data obtained from individuals within the same family. The advantage of this approach is that family size and clustering within families is also taken into account compared to the lambda calculation where only one affected family member is included in the calculation. The authors report that both methods yielded similar results.

The authors report that the presence of dementia was only slightly increased among siblings and parents of patients with sporadic ALS and was not significantly increased in relatives of patients with familial ALS. It is surprising that an increase in the rate of dementia was not seen in relatives of patients with FALS as the C9orf72 repeat expansion is found in almost 50% of familial cases of ALS and is associated with dementia (FTD). This may be due to inadequate power, or methodological issues relating to the fact that patients with cognitive impairment self-reported the presence or absence of dementia in their relatives.

There was no significant difference in the rate of PD between relatives of cases and relatives of controls. There was, however, significantly less cardiovascular disease in relatives of patients compared with relatives of controls.
In summary, only three of the seven studies identified had the primary aim of investigating family history among relatives of patients with ALS. These family aggregation studies do report an increased rate of dementia among family members of patients with ALS, though few results reach statistical significance because power is small. Some studies also note that the distribution of the dementia cases was uneven and tended to cluster in certain families. All studies were carried out before the discovery of the C9orf72 repeat expansion, which is associated with ALS and FTD. To date no aggregation study has been carried out looking at risk of aggregation in ALS patients depending on the presence of absence of C9orf72. It is fair to speculate that relatives of C9orf72 repeat expansion carrying ALS patients maybe at higher risk of developing disease than relatives of ALS patients without this gene.

1.7.3 Single case reports of aggregation of other conditions in patients with ALS and their family members

A number of papers were reported where patients with ALS developed signs and symptoms of another disease. In 1885, Westphal[161] reported two patients presenting with a protracted course of ALS and who subsequently developed psychosis. A further case report[162] in 1922 detailed a patient with ALS who developed hallucinations and paranoia. None of these patients were reported to have a family history of ALS or psychosis. The first report of a patient with schizophrenia developing ALS was published in 1941[163]. This patient did have a family history of schizophrenia. Nothing further appeared on the topic until 1981, when Burnstein and colleagues[164] described an extensive pedigree with ALS, dementia, epilepsy and schizophrenia/psychosis appearing in family members across three generations.

It is possible that some of the syndromes described in these early papers correspond to co-morbid FTD rather than psychiatric disorder. A recent work by Lillo and colleagues[165] identified the presence of delusions in half of all patients with ALS-FTD and so it may be argued that these reports from the end of the nineteenth century are in fact the first reports of frontotemporal dementia co-morbid with ALS, however this link between psychiatric disease and ALS should not be overlooked.
One small study from 1994 compared the pedigrees of twelve Schizophrenic Ashkenazi Jews to seven healthy Ashkenazi Jews and found that the rate of Schizophrenia, Tay-Sachs disease, Gaucher disease, and ALS was increased among relatives of the schizophrenic probands[166]. The rate of ALS was 155 times higher than expected (3/974), although the sample size was small, and the authors could not establish whether some cases of adult Tay-Sachs had been misclassified as ALS. Given that the lifetime risk of ALS is estimated to be 1 in 350 in men and 1/472 in women (average 1/411) [95, 96], it is difficult to understand how a rate of 3 in 974 cases (1 in 325) generates an OR of 155. Interestingly, the authors report that all adult cases of Tay-Sachs disease, an autosomal recessive disorder that may present in a similar way to ALS with upper and lower motor neuron dysfunction, present with psychotic symptoms.

**Clustering of ALS-Parkinsonism-Dementia complex**: An increased incidence of ALS been reported in Guam, and on the Kii peninsula of Japan[21]. The phenotype was particular to the regions of Guam and Kii, and patients demonstrated a constellation of symptoms including Parkinsonism, dementia, and features of ALS. The incidence of ALS in Guam was almost three times as high as that seen in other countries, and the age of onset was decades earlier, however the incidence dropped between the 1950's and more recent time, leading investigators to the conclusion that Cycad exposure was to account for the condition. There is still considerable debate over whether this epidemic was due to a genetic founder effect or to the neurotoxic effects of Cycad ingestion[167]. This debate is fueled in part by a recent paper that describes the presence of C9orf72 mutations among 20% of ALS patients from a cluster in the Kii peninsula[168]. To date, no reports on the rate of C9orf72 in Guam are published. Of note an epidemiological survey of schizophrenia and chronic mental illness in Micronesia from the 1980’s noted that the rate of schizophrenia and chronic psychiatric illness was much higher in Guam than in surrounding parts of Micronesia[169].

In summary, case reports of family aggregation of ALS and psychiatric illness are few and studies of the family aggregation of neuropsychiatric diseases in ALS are non-existent.
1.7.4 Aggregation of other psychiatric conditions in families

To further investigate the possibility that psychiatric conditions may aggregate in families of ALS patients and could potentially share a genetic susceptibility locus a further review of the literature was undertaken to identify other neurological conditions associated with psychiatric diseases.

**Huntington's disease (HD):** is a fully penetrant autosomal dominant disorder, caused by a pathological trinucleotide expansion on chromosome four. Production of the protein Huntingtin leads to an accumulation of protein, and abnormal protein-protein interactions. Patients present in a number of different ways; most commonly with the choreiform movements that are characteristic of the condition, or with psychosis, personality change and/or dementia.

The eponymous Huntington was the first to recognize the existence of psychiatric disease both in patient with chorea and also in their relatives without chorea.

> "The tendency to insanity, and sometimes that form of insanity which leads to suicide, is marked. I know of several instances of suicide of people suffering from this form of chorea, or who belonged to families in which the disease existed"

*George Huntington, 1872*

Tsuang and colleagues[170] reported the occurrence of psychosis in family members of 22 HD patients with psychosis and 22 HD patients without psychosis. Patients in both groups had an equal number of CAG repeats (mean 47.5) but the rate of psychosis in the relatives of HD cases with psychosis was increased five-fold (p=0.02). Most of the relatives also had been diagnosed with HD.

Maio and colleagues[171] reported an increased rate of suicide among 2,793 HD patients and their relatives. As this was carried out prior to the discovery and commercial introduction of testing for the CAG repeat they classified relatives as 50% at risk, 25% at risk, and not at risk, depending on the degree of relatedness to the affected individuals. They found that the rate of suicide was elevated in all groups compared with the US reported rate, and that the rate was
proportional to the personal risk of developing HD. The group classified as 'not at risk' also had an increased five-year rate of suicide (3.2%).

**Frontotemporal dementia (FTD):** Schoder and colleagues[172] recently reported a family aggregation study, which demonstrated an increased morbid risk of schizophrenia in relatives of patients with FTD compared to relatives of patients with AD. They reported a relative risk of schizophrenia of 4.1 in 741 first-degree relatives of 100 FTD patients. While FTD and schizophrenia are both neurobehavioural syndromes characterised by frontal dysfunction and abnormalities of social interaction, the two differ in that schizophrenia is characterized by a markedly younger age of onset, relapsing and remitting course, prominent hallucinations and delusions, whereas FTD presents with an older age of onset, progressive decline, and psychosis reported rarely. Because of overlap between the syndromes of FTD and schizophrenia, the authors interviewed each relative with schizophrenia, wherever possible, to confirm that they were not a misdiagnosed case of FTD. They also reported three families where schizophrenia and FTD segregated with PRGN and VCP mutations. The study by Campion was published prior to the discovery of the C9orf72 repeat expansion, which is associated with one-third of cases of FTD, so it is not known whether relatives of FTD cases with the C9orf72 repeat expansion have a higher rate of schizophrenia.

This is an important study – the strong association between ALS and FTD both in individual patients and other family members has been recognized. Association of schizophrenia and FTD and segregation with VCP mutations are compelling evidence that a family aggregation study of psychiatric disease in ALS needs to be undertaken.

### 1.7.5 Methodology used in family aggregation studies

Family aggregation studies identify groups of individuals with a specific phenotype (e.g ALS) and apply epidemiological methods to determine whether the disease occurs at a higher rate among their relatives compared with a matched control population. If the phenotype of interest is a disease then the trait is dichomotomous (affected/unaffected). It is also possible to study a physiological trait with a continuous outcome (e.g blood pressure).
Two study designs for collecting family aggregation data are available to the researcher: the cohort study and the case-control study.

The cohort study identifies at-risk individuals who are currently free of disease and in the follow-up period, assesses risk factors that are associated with the development of disease. This study design is less subject to selection bias than the case-control method but, in the case of a rare disease, such as ALS, where the lifetime risk for ALS is estimated to be 1 in 350 in men and 1/472 in women [95], thousands of healthy people would need to be studied to encounter even five or six cases of ALS during the follow-up period.

The case-control study overcomes this problem by identifying a representative sample of cases, ideally using a population based register, and comparing these to disease-free controls. Once a study design is decided, family medical information is collected. There are two methods available for collecting family information – the family history method and the family study.

In the Family History Method, the proband answers questions about the health of their relatives[173]. An abbreviated family history may be taken by enquiring about the presence of specific diseases in relatives, without clarifying further details of the illness or demographic factors. A detailed family history, where the researcher collects as much information on as many family members as possible, including specifics regarding any disorders identified (e.g. age at onset etc), demographic details, and information necessary to verify medical conditions reported (e.g. date of death for death certificate verification), may also be taken. Clarification of the history from more than one family member is advisable, particularly if the proband has evidence of cognitive impairment. This method makes possible the collection of information on large numbers of relatives. Provided histories are properly verified using independent sources (e.g. death certificates, hospital records), this is a very reliable method for collecting family aggregation data.

The Family Study Method differs from the family history method in that, in this case, each member of the proband’s family is contacted directly to enquire about health status[173]. This method ensures that the information collected is accurate as each person reports his or
her own medical history directly to the researcher. If features of the condition under study (e.g. tremor in a study of aggregation of PD) are reported then the researcher arranges to visit the person and verifies the diagnosis by clinical examination. This method is very labour intensive and expensive to perform compared with the family history method. Patients may also be reluctant to enter into a study if every living member of their family is to be contacted. Elbaz and colleagues[174] report a large family aggregation study, from the Mayo Clinic, which compared families of PD sufferers and family members of controls. They carried out subgroup analysis on a cohort where both the family history, and family study method had been undertaken to compare the accuracy of the two methods. They found that the sensitivity and specificity of information collected by the family history method was 70% (95% CI 51-84%) and 99% (95% CI 98-99) respectively compared with the family study method. The family study method was substantially more expensive and required many more resources to complete in comparison to the family study method which was cheaper and quicker. The family history method also allowed the collection of data on many more relatives than in the family study. The family history component of this study did not acquire medical notes or death certificates to confirm diagnoses reported by relatives.

1.7.6 Analytical methods used to describe the results from family aggregation studies

Once the information has been collected from hundreds of families, the data is analyzed. Data management is extremely important as a study involving 200 cases and 200 control families can generate information on over 15,000 relatives. A number of different analytical methods can be used to assess the data and the pros and cons associated with each method are discussed below.

**Relative risk:** Relative risk of the disease in question, lambda (λ), is calculated by dividing the rate of disease among relatives of patients with ALS by the rate of disease among relatives of controls. Confidence intervals can also be calculated.
\[ \lambda = \frac{\text{Number of cases reported}}{\text{Number of ALS relatives included in study}} \]  
\[ \frac{\text{Number of cases reported}}{\text{Number of control relatives included in study}} \]

*Equation 2 Relative risk calculation*

This is the accepted method for analysis of the difference in incidence between groups. All seven of the family aggregation studies discussed earlier in the chapter used this method of analysis. The major benefits of this type of analysis is that it is quick to perform and the mathematics are simple. One drawback of this type of analysis is that bias can be introduced if like individuals are not compared with matched comparators. As first-degree relatives share 50% of their genetic data and third-degree relatives only share 12.5% of their genetic information, initial analysis must compare first-, second- and third-degree relatives to each other directly or, alternatively, they must account, in the denominator, for the proportions of the degrees of relatedness used. If the rate is the same across degrees of relatedness then the numbers may be combined for more power.

The second thing to consider is that if one is collecting data on second-degree relatives it will include nieces/nephews, aunts/uncles and grandparents. Not all groups will have reached the age of risk so if one group has a higher proportion of grandparents then results may falsely demonstrate a higher rate of conditions associated with older age. This can be addressed in a number of ways, most effective of which is to compare people within the corresponding groups (eg grandparents only), but this results in less power. Proportional comparisons, which take into account the number of cases of nieces/nephew compared to grandparents in each group, can also be carried out, and if the age of each individual is known then analysis based on cumulative lifetime risk can be carried out (described below).

Calculation of relative risk is most often used when authors only have the number of affected individuals within a cohort but do not have the ages at which the individuals exit the study.
through death, develop of the condition in question, or are well at the time of the study (censoring). If ages are unavailable then the years-at-risk cannot be controlled for in the analysis. For a thorough investigation of relative risk the age at censoring is essential.

**Cumulative incidences:** Survival analysis methods and cumulative lifetime incidences are frequently used to control for differences in the years-at-risk for each disorder. The survival analysis method is particularly useful in studies looking at disorders with later onset, where some family members are too young to have expressed the disease phenotype, and other relatives may have died of another cause before manifesting the disorder. This method is also preferable as second degree relatives may be grandparents, aunts/uncle and nieces/nephews: if the proportion of nieces/nephews is higher in the case group compared to the control group then this may bias the results as the cohort will be younger and less likely to have developed the disease under consideration. Another benefit of the survival analysis method is that the age of expression of the phenotype can be accurately compared between groups. For example, one would expect that individuals with more risk factors for dementia would develop it at an earlier age than members of the general population.

Cumulative incidence estimates calculate the risk to a relative of an ALS patient manifesting the condition by a particular age when compared with the relative of a control. These results can be represented graphically on a cumulative incidence 'hazard rate' plot, which is the inverse of a Kaplan-Meier plot. Cox proportional hazards analysis is used to calculate the rate ratio for each disorder in relatives of ALS patients versus controls, after correcting for other factors such as degree of relatedness, sex etc.
1.8 Common themes in neurodegenerative and neuropsychiatric disease

1.8.1 Introduction

Section 1.7 described a number of family aggregation studies of ALS that demonstrate the aggregation of other diseases, in particular neurodegenerative diseases, in families of patients with ALS. A number of case reports reviewed in the literature search also described concomitant psychiatric disease in families of patients with ALS.

This section explores the biological, histopathological, and molecular genetic parallels which may explain why aggregation of different conditions occurs within a single kindred. Many conditions may share a common pathological pathway, which later differentiates into a distinct phenotype.

1.8.2 Common themes: Clinical features of neurodegenerative disease

The term 'neurodegenerative condition' refers to age-dependent progressive diseases, caused by degeneration of the central nervous system (CNS). Neurodegenerative diseases share certain common features including clinical course, histopathology, and molecular mechanisms of pathogenesis. When comparing two different diseases, there may be both overlap with regard to some features and divergence of other features. As new categories of disease emerge, some are seen to share common pathogenic features and genetic origins. Common clinical features of neurodegenerative disease are listed below in table 1.5.
Common clinical features of neurodegenerative disease

- The clinical course is chronic and relentless until death
- The disorder is not reversible by any known drug therapy
- Phenotypic variation is commonly seen, even within families
- Cognitive impairment and dementia are common features
- The major risk factor is advancing age
- The condition appears to be heritable in a small percentage of cases
- In the familial form of the disease the age at onset occurs up to a decade before onset in sporadic cases
- Several different neurodegenerative conditions may appear within the one family
- Features of more than one neurodegenerative condition may co-exist in one person

Table 1.5 Common clinical features of neurodegenerative diseases

Eponymous classifications (e.g. Alzheimer disease, Parkinson disease, and Huntington disease) remain useful in a clinical setting, as the diagnosis generates a framework for clinical discussion, prognostication and disease management. However, it is increasingly recognized that neurodegenerative diseases can also be subdivided into categories based on pathological or genetic characteristics, as outlined in the figure 1.2.
Clinical Classification of Neurodegenerative Diseases

Predominantly Cognitive Symptoms
- Amnestic
  - Alzheimer's dementia
  - Non-Alzheimer dementia
  - FTD complex
    - FTD-tau (tau +ve, ubiquitin +ve/-ve)
    - FTD-ubiquitin (tau -ve, ubiquitin +ve)
    - TDP-43 +ve
      - Atypical FTD-ubiquitin

Predominantly Motor Symptoms
- Executive dysfunction
  - Parkinson’s disease
  - Trinucleotide repeat disorders
    - Friedreich’s Ataxia
    - Huntington’s disease
    - Spinocerebellar ataxias
  - Motor Neuron Disease
    - PLS
    - PMA
    - ALS

Weakness
- Movement disorder
- Ataxia

1.8.3 Common themes: Neuropathology

A number of common themes have emerged in the pathogenesis of various neurodegenerative diseases. These pathological processes may reflect common upstream mechanisms, or alternatively they may reflect the fact that neurons have a limited number of ways in which to die[175]. Regardless of the underlying mechanism many neurodegenerative disorders are associated with protein aggregates (table 1.6).
<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein deposited</th>
<th>Site of deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson's disease</td>
<td>α(alpha)-synuclein</td>
<td>Intracellular (Lewy neuritis and Lewy bodies)</td>
</tr>
<tr>
<td>Multiple systems atrophy</td>
<td>α(alpha)-synuclein</td>
<td>Intracellular argyrophilic inclusions in both oligodendroglia and neurons</td>
</tr>
<tr>
<td>Hereditary cerebral amyloid angiopathy</td>
<td>Cystatin C</td>
<td>Extracellular</td>
</tr>
<tr>
<td>Congophilic amyloid angiopathy</td>
<td>Aβ(beta)</td>
<td>Extracellular</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>Aβ(beta)</td>
<td>Extracellular (amyloid plaques)</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>Tau</td>
<td>Intracellular (paired helical filaments)</td>
</tr>
<tr>
<td>Frontotemporal dementia</td>
<td>Tau TDP-43</td>
<td>Intracellular inclusions (Have either Tau or TDP-43 inclusions)</td>
</tr>
<tr>
<td>Familial British dementia</td>
<td>Aβri</td>
<td>Extracellular (amyloid plaques)</td>
</tr>
<tr>
<td>Familial Danish Dementia</td>
<td>ADan</td>
<td>Extracellular (amyloid plaques)</td>
</tr>
<tr>
<td>Transmissible spongiform encephalopathies</td>
<td>Prion protein</td>
<td>Extracellular amyloid plaques and/or diffuse deposits</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>TDP43-ubiquitin</td>
<td>Cytoplasmic inclusions and ubiquitin positive neuronal threads</td>
</tr>
<tr>
<td>Huntington 'sdisease</td>
<td>Mutant huntingtin</td>
<td>Nuclear and cytoplasmic inclusions</td>
</tr>
</tbody>
</table>

Table 1.6 Neurologic diseases associated with aberrant protein structure

The observation that ALS and FTD can occur within members of the same family, and also in the same individual, led to the discovery that TDP-43 protein aggregates were common to both conditions[176]. These findings lead researchers to speculate that TDP-43
proteinopathies may have common pathological process that can lead to a clinically diverse spectrum. The means of differentiation is not currently understood.

There is also evidence that protein aggregation may also underlie psychiatric disorders such as schizophrenia. Studies looking at DISC1 mutations in schizophrenia have identified that abnormal protein signaling may result in deleterious protein aggregation. Postmortem studies of people with schizophrenia have identified an increase in DISC1 insoluble aggregates in brain tissue[177].

Increased branching of subterminal motor neurons is seen in patients with schizophrenia. On electrophysiological studies, abnormalities of the alpha-motor neuron and motor unit, consistent with denervation and reinnervation, have been demonstrated in patients with schizophrenia and their first-degree relatives[178-180]. Various neuropathological findings have been described in schizophrenia which may suggest a common link with the pathological process in ALS[181].

1.8.4 Common themes: Genetics

Familial aggregation of specific neurodegenerative diseases has been discussed in the previous literature review. Apart from the trinucleotide repeat disorders, which exhibit Mendelian inheritance with full penetrance and anticipation, neurodegenerative disorders are mostly apparently sporadic, with a small proportion of familial cases. Sporadic and familial cases are usually phenotypically and histologically indistinguishable, although the symptom onset in familial cases tends to occur at an earlier age. This suggests that genetic mutations, with or without a higher burden of environmental risk, accelerate the molecular processes that lead to late-onset sporadic disease.

A number of causative genes have been discovered for specific neurodegenerative diseases that influence protein aggregation. Mutations in APP, Presenilin 1 and Presenilin 2 which occur in early onset AD cause altered protein production and increased aggregation of β-amyloid protein[182]. Similarly PARK 1, the first gene to be identified in PD, alters the production of the protein α-synuclein[183]. Mutations in genes associated with oxidative stress pathways, SOD1 and DJ1, have been implicated in familial ALS and PD, respectively.
The discoveries of *TARD-P* and *FUS* in ALS and ALS-FTD have suggested a role of altered RNA regulation in some neurodegenerative diseases[184].

Genome-wide association studies and high-throughput sequencing have identified susceptibility genes in many neurodegenerative conditions. Overlap between susceptibility genes has also been reported. *APOE4* is well established as a risk factor for late-onset AD[185], and meta-analysis has shown that presence of the allele is also linked to PD and FTD[186]. Although the incidence of *APOE4* is the same in patients with ALS as the general population, the presence of the *APOE4* allele is associated with earlier age of disease onset[187].

A recent paper using genome-wide association methodology reported that a higher frequency of *ANG* variants was found in patients with ALS and in patients with PD, indicating that the *ANG* variant may lead to abnormal processes in a common neurodegenerative pathway[137].

Perry syndrome is a rare autosomal dominant disorder caused by a mutation in the Dynactin gene, *DCTN1*, which presents with features of parkinsonism, behavioural change, and marked depression with suicidal thoughts, with death within five years of onset[188]. The exact biochemical mechanism leading to presentation with parkinsonism and psychiatric problems is unknown, but interference with normal axonal transport and aggregation of *TDP-43* is believed to play a role. Dynactin mutation may also increase susceptibility to ALS[189].

A striking observation is the fact that a single mutated gene can cause such a wide variation in phenotype expression. In Mendelian diseases, differences can be evident within families with the same mutation. A mutation in *INF2*, known to cause glomerulonephritis, can also cause Charcot-Maire-Tooth Disease with glomerulopathy[190]. *INF2* encodes a protein involved in essential steps of myelination and it can affect both neural and glomerular myelination. In families with a genetic mutation that causes ALS the phenotypic variation can be marked - one member may develop a rapidly progressive bulbar form of the condition with an early onset, and die within months while another family member may have a slowly progressive indolent flail-arm form of the disease, later onset, and live many years with the
condition. Patients carrying a pathological expansion in \textit{C9orf72}, can develop either ALS, FTD or ALS-FTD. At present it is unclear whether repeat number influences phenotype expression. At present it is unknown if the phenotypic variations result from a single gene mutation, the presence of two genes of direct effect (oligogenic theory), are due to an intrinsic genetic mechanism, the interplay of other protective and pathological genes, or the influence of environmental factors on the genetic expression. What is known is that the same mutated gene can cause huge phenotypic variation and for this reason family aggregation studies are very important in the search for new causative genes.

\textbf{1.8.5 Common themes: Biochemical processes}

\textbf{Selective Vulnerability:} Neuronal cells are constantly placed under stress because of intrinsic metabolic processes and also because of a number of extrinsic environmental factors. Neurons are vulnerable as they facilitate neurotransmission and maintain the high metabolic needs required for long axonal projections. Larger neurons with myelinated axons extending long distances appear to be most vulnerable. These neurons have high energy requirements, are especially reliant on axonal transport and have a larger surface area exposed to environmental toxins. In addition to the fact that once damaged they cannot regenerate, neurons in general represent a vulnerable group of cells[175].

\textit{Neuronal Vulnerability}

\begin{itemize}
  \item \textbf{Intrinsic Factors}
    \begin{itemize}
      \item i) Highly active metabolic processes.
      \item ii) High energy requirement compared to other cells.
      \item iii) High demand for ROS as signaling molecules.
    \end{itemize}
  \item \textbf{Extrinsic Factors}
    \begin{itemize}
      \item i) Long axonal projections.
      \item ii) Large surface area for exposure to environmental toxins.
    \end{itemize}
\end{itemize}

\textit{Figure 1.3 Factors leading to neuronal vulnerability}
Selective susceptibility of certain neurons to similar pathological processes results in particular disease phenotypes.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Vulnerable neuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Upper and lower motor neurons</td>
</tr>
<tr>
<td>Alzheimer disease</td>
<td>Cholinergic neurons</td>
</tr>
<tr>
<td>Parkinson's disease</td>
<td>Dopaminergic neurons</td>
</tr>
<tr>
<td>Frontotemporal dementia</td>
<td>Frontotemporal cortical neurons</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>GABAergic neurons</td>
</tr>
</tbody>
</table>

*Table 1.7 Neurologic diseases and selectively vulnerable cells*

In ALS, motor neurons are primarily affected, although it is now recognized that non-motor neurons are involved in cognitive dysfunction. Motor neurons are large cells and often have long axonal fibres. In lower motor neurons these fibres may stretch over a metre in order to supply distal muscles. They require a strong cytoskeleton, neurofilament network and efficient axonal transport system.

*Disrupted Axonal Transport*

\[ \text{Kinesin Mediated Anterograde Transport} \]

SOD1 aggregates may lead to retrograde axonal dysfunction leading to:

i) Toxicity at synapses
ii) Loss of positive feedback
iii) Mitochondrial disruption
   leading to decreased energy

\[ \text{Dynactin Mediated Retrograde Transport} \]

*Figure 1.4 Disruption of axonal transport*
Motor neurons are highly metabolic and exquisitely sensitive to energy demands. Deficiencies of protective agents, such as glutathione and cytosolic calcium binding proteins, as is the case in the substantia nigra, can add to neuronal stress.

**Aberrant protein formation:** The formation of aberrant structures by many different proteins appears to underlie a large number of diseases, many of which afflict the CNS. The mechanism by which the attainment of an aberrant protein structure causes disease is still unclear and may involve both the loss of a vital physiological function, and the acquisition of pathological properties. Accumulation of aberrant proteins may strain the normal mechanisms responsible for controlling protein folding and degradation resulting in a generalized loss of protein homeostasis and consequent toxicity.

It has been suggested that a threshold of abnormal aggregation must be reached before clinical signs appear, but it is as yet unclear whether deposited protein aggregates or other smaller protein assemblies are the principle mediators of disease. Evidence against deposits of protein aggregates as mediators of disease comes from the finding that many aggregated proteins are found in brains of elderly individuals who die without clinical signs of disease.

There is a belief that abnormal protein aggregates in the brain may cause a 'wildfire' effect, and cause the contiguous spread of abnormal pathways in an anatomical region[191].

Inclusion bodies are relatively large electron-dense structures that contain membrane-limited protein aggregates, and are seen in many brain disorders. Such deposits often contain ubiquitin-positive material, which is believed to accumulate due to impairment of the ubiquitin-proteasome system. Under normal conditions cytoplasmic proteins are tagged for destruction by the enzymatic addition of four or more ubiquitin molecules, but build-up of substrate, decreased efficiency of ubiquitin conjugation or impaired degradation of ubiquitinated protein can trigger accumulation of partially ubiquitinated protein aggregates[192].

Primary TDP-43 diseases of the nervous system include ALS with and without FTD, FTD alone, Inclusion body myositis with Pagets disease and frontal dementia (IBMPFD), and Perry disease. Other diseases of the brain with secondary TDP-43 pathology include the
parkinsonism-dementia-ALS complex of Guam, Alzheimers disease, Parkinson’s disease, and Huntington’s disease[193].

In all these cases the protein aggregation product is the same, and the clinical phenotype depends on the region of protein deposition; for example TDP-43 deposition in the frontal lobes would lead to FTD, and in the anterior horn cells of the spine would lead to ALS. The processes which drive the location of protein deposition are unknown.

FUS/TLS protein aggregation are found in ubiquitin deposits of ALS patients that are negative for TDP-43.

**Altered RNA metabolism:** There is increasing interest in the role of aberrant RNA processing in the pathogenesis of neurodegenerative disease. Mutations in two important genes, FUS and TDP-43, identified in a small percentage of familial ALS and FTD cases, are most widely recognized as a cause of abnormal RNA processing in ALS. Mutations in the TAR DNA binding protein, TDP-43, and the protein FUS/TLS have widespread downstream effects on multiple differentially spliced mRNA species[184]. Consequently, it is anticipated that quite diverse pathogenic pathways are triggered depending on the RNA binding protein and the type of neurons involved[194]. Other ALS genes, including SETX, are also implicated in abnormal RNA processing. Loss of function mutations in another RNA regulator, PRGN, has been linked with FTD and schizophrenia[172].

**Oxidative Stress:** In all cells, but particularly highly metabolically active cells such as neurons, there is a constant production and elimination of reactive oxygen species (ROS). At any time the balance is such that an unusual increase in ROS or loss of antioxidant protection can lead to accumulation of ROS and ensuing cellular damage. High levels of ROS can cause nuclear DNA oxidation and repairing such damage requires substantial expenditure of metabolic energy. If damaged DNA is not adequately repaired this can lead to cellular dysfunction and apoptosis. Accumulated oxidatively damaged DNA has been observed in AD, PD, ALS, and vascular dementia (VD). Calcium plays an integral role in signaling within the cell and also in maintenance of cellular homeostasis. As part of the role in signaling, ROS activate calcium channels and deactivate calcium pumps. This leads to abnormally high
intracellular levels of calcium, which in turn may lead to cell death. Mitochondrial ROS also cause increased uptake of calcium ions with increased membrane permeability, resulting in the release of cytochrome-C, which initiates the apoptotic cascade[195].

In vivo and in vitro studies have shown that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase derived from microglia play an important role in the generation of ROS. In PD, microglia-specific NADPH oxidases are involved in the production of ROS, which may contribute to the death of dopaminergic neurons. A similar process is seen in ALS; whereby oxygen radicals produced by microglial NADPH oxidase are believed to injure motor neurons. Evidence from the mutant SOD1 mouse model of ALS indicates that genes encoding NADPH oxidase are upregulated in disease and this leads to an increased concentration of ROS in mouse spinal cord tissue.

**Mitochondrial dysfunction:** Mitochondria play a crucial role in the production of cellular energy using the respiratory chain. Consequently, accumulated mitochondrial dysfunction is implicated in both normal ageing and neurodegeneration. Proposed mechanisms of this effect include failure to meet the energy needs of the cell, calcium misregulation leading to cell death, over production of ROS and cytochrome C-induced apoptosis[196].

![Mitochondrial Dysfunction]

*Figure 1.5 Mitochondrial dysfunction is implicated in ageing and degeneration*
Studies into psychiatric disease have demonstrated that cluster analysis can differentiate patients with schizophrenia from controls because of impaired mitochondrial function in patients [197].

Well-documented incidents have shown that ingestion of certain neurotoxins which inhibit mitochondrial pathways can lead to the sudden onset of clinical syndromes identical to neurodegenerative diseases such as PD or HD.

D-pramipexole, currently under phase three trial for ALS, is thought to be beneficial in ALS as it increased the efficiency of mitochondria. Recent research suggest that the mode of action may include maintenance of energy production in the mitochondria of affected motor neuron cells[198].

**Excitotoxicity:** Glutamate is the primary excitatory neurotransmitter in the CNS and its dysregulation has been implicated in the development of neurodegenerative diseases and schizophrenia. Glutamate has essential roles in synaptic transmission and plasticity, which are important in learning and memory as well as sensory and motor functions. Transmission of glutamate is mediated through three major receptors—N-methyl-d-aspartate receptors (NMDA), α(α)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainite receptors. The glutamate excitotoxicity hypothesis postulates that excessive synaptic glutamate causes over-activation of the post-synaptic NMDA and AMPA receptors resulting in neuronal death. High glutamate levels, which continuously activate post-synaptic receptors, may lead to increased intracellular calcium and catabolic enzyme activity. Downstream effects can include depolarization of mitochondrial membrane, activation of the caspase system and production of reactive oxidation species, all of which culminate in cell death[199].
Excitotoxicity

Figure 1.6 Proposed mechanisms for excitotoxicity

Excessive synaptic glutamate may be potentiated because of a fault in the cellular glutamate re-uptake system. Excitatory amino-acid transporter 2 (EAAT2) is a glutamate transporter involved in cerebral glutamate transport. It has been postulated that some patients with ALS have decreased expression of this protein. Similar studies carried out in patients with AD have also shown a reduction in EAAT2 expression. It has been shown that GLUR2, an AMPA glutamate receptor subtype responsible for calcium permeability into the post synaptic cell, is not expressed in motor neurons affected by ALS because of a defect in the editing process for messenger RNA encoding the GLUR2 receptor. Absence of a functional GLUR2 subunit allows calcium influx into the postsynaptic cell and results in cellular damage.

In schizophrenia and affective disorders there are problems with regulation of dopamine and glutamate.

In PD, parkin, the protein product of PARK2, has regulatory effects over excitatory glutaminergic synapses. Abnormalities in parkin production can lead to enhanced synaptic activity and may even trigger an increase in the number of glutamate receptors. Excessive glutaminergic activity may be responsible for nigral excitotoxicity.

Given this evidence, down-regulation of glutamate activity may be a potential therapeutic target in neurodegenerative disease. Although the exact mechanism is unknown, riluzole is
believed to work by blocking glutamate receptor activation in ALS. No other anti-glutamate agent has been successful in disease treatment[200].

**Neuroinflammation and Microglial activation:** An epidemiological study carried out in 1980s was the first to postulate an association between inflammation and neurodegeneration[201]. The study demonstrated that the incidence of AD was lower in patients with rheumatoid arthritis (RA) who had been on long-term anti-inflammatory treatment than those who had not. Since then, detailed descriptions of systemic and CNS specific pro-inflammatory cascades have fueled the hypothesis that neuroinflammation plays an active role in the process of neurodegeneration. Microglia are CNS-specific macrophages, derived from myeloid precursor cells, which enter the CNS during embryogenesis. The primary function of this subset of immune cells is to protect the brain from extrinsic pathogens and processes. Recently, a number of experiments have shown that activated microglia can cause irreversible damage to tissues of the CNS. During the process of activation, microglia are highly plastic and differ in morphology and phenotype depending on the nature of the insult causing activation. Microglia may remain in the activated state for prolonged periods. Neuroinflammation is found in both ALS and PD: work done on chimeric mice has demonstrated microglia and infiltrating T at the site of neuronal injury[202]. However, it is unlikely that microglial activation is the primary cause of any neurodegenerative process. It is more likely that an initial challenge induces an inflammatory cascade, which in turn initiates maladaptive processes and positive feedback loops that cause further pathological inflammation.
Postmortem examination of brain tissue from PD patients has demonstrated activated microglia in the substantia nigra pars compacta. In AD, neuroinflammation is considered a downstream effect of abnormal protein production. Aβ(beta) causes up-regulation and activation of microglia leading to an inflammatory cascade. This cascade sets out to respond to abnormal protein accumulation but causes damage as a byproduct of activation.

Inflammation is also implicated in Schizophrenia. A GWAS study of patients with Schizophrenia identified an association with the Major Histocompatibility Complex locus[203].

While several known pathophysiological processes make neurons vulnerable to degeneration, we still do not know fully why some people are more susceptible to specific diseases, and why different diseases cluster within some kindreds. Family members within a kindred may share genetic factors that result in dysfunction of similar common pathways, but other factors may cause differentiation of the pathological process into a number of clinical end-phenotypes, thus leading to aggregation of discrete conditions within the same family (see figure 1.8).
Healthy individuals

Common pathological processes mediated by genetic and environmental factors include:
- Selective vulnerability
- Aberrant protein formation and aggregation
- RNA metabolism abnormalities
- Oxidative stress and mitochondrial dysfunction
- Excitotoxicity
- Inflammation

Occult factors such as genes, gene-gene interactions, environmental factor, and gene-environment interactions which differentiate common pathological processes into particular disease phenotypes

Individuals with different disease phenotypes

Figure 1.8 Common pathways leading to different clinical phenotypes

1.9 Summary

Chapter 1 comprises a detailed review of the current understanding of the ALS phenotype, and the epidemiology, treatment and aetiology of the disorder, particularly the genetic aetiology. I have highlighted potential gaps in the literature of familial ALS and the aggregation of other disorders among relatives of ALS patients.

<table>
<thead>
<tr>
<th>Accepted rate of FALS</th>
<th>Rate of FALS predicted to occur by chance</th>
<th>Rate of FALS in family aggregation study</th>
<th>Rate of family history of ALS in healthy controls</th>
<th>True rate of FALS (actual rate minus background rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

85
Chapter 2  Aims

2.1 Familial Amyotrophic Lateral Sclerosis

**Definition of FALS:** Despite thousands of articles written about familial ALS, is unclear from review of literature whether a standard definition exists for the term FALS. A standard definition for FALS is crucial to allowing comparisons to be made between different regions and also for inclusion of familial cases in genetic studies.

Specific aims:

1. To determine whether a consensus for the term 'FALS' exists among clinicians
2. To propose criteria for Familial ALS

A questionnaire based study design was chosen to identify if a consensus for the definition of the term familial ALS exists among neurologists and researchers.

**Rate of FALS in the literature:** As discussed in the previous chapter the rate of familial ALS is believed to be 10%. However, the reported rate of FALS from clinical studies is variable across regions and across time periods and often does not reflect the currently accepted rate of 10%. No meta-analysis of the reported rate of FALS has ever been carried out.

Specific aims:

1. To determine the reported rate of familial ALS among published population-based studies

The rate of reported familial ALS among published population-based studies was estimated by performing a systematic review of the literature and meta-analysis.

2.2 Family aggregation study

To main aim is to test the hypothesis that aggregation of ALS, neurodegenerative diseases and neuropsychiatric disorders occur to a higher degree among family members of people with ALS compared to relatives of controls.

The primary role of aggregation studies is to identify conditions that cluster in families. Such a method can apportion risk to other family members and may also inform future genetic studies.
Aggregation of ALS: The true rate of familial ALS is unknown. From the literature it is clear that a number of cases of familial ALS may be missed when a newly diagnosed patient is being recruited to a population-based register. The exact number is unknown. In order to determine the true rate of familial ALS, including those familial cases that at present are not reported for the reasons discussed, a detailed population-based family aggregation study is necessary with appropriate genealogical methodology, and verification of cause of death to capture all ALS cases among relatives.

The risk to relatives of ALS patients, of themselves developing the disease, has not been estimated for the presence or absence of the C9orf72 repeat expansion in the ALS patient

Specific aims:

1. To determine the true rate of familial ALS in a population-based study
2. To determine the risk to relative of ALS patients of developing ALS, and to stratify this risk according to the absence or presence of the C9orf72 repeat expansion
3. To use information collected to direct future genetic studies

These aims were achieved by designing and carrying out a family aggregation study. Incident ALS patients from the four-year period 2008-2011 were identified using the Irish ALS Register and detailed family history taking techniques and genealogical methods were used to identify all cases of ALS in the families of a representative portion of incident ALS patients. The same data was collected from matched controls. Comparisons are made between cases and controls including family history, epidemiological data, family structure and size, and fecundity. Large pedigrees, negative for known genes are considered for future genetic studies.

Aggregation of neurodegenerative and psychiatric disease: The rate of aggregation of neurodegenerative diseases among relatives of patients with ALS has been reported in seven previous family aggregation studies, however none of these studies stratify for the presence or absence of the C9orf72 repeat expansion. Although case studies exist for the aggregation of psychiatric disease in the families of ALS patients, no family aggregation studies exist looking at this.
Specific aims:

1. To determine the risk to relatives of ALS patients of developing other medical conditions including neurodegenerative and psychiatric condition, and to stratify this risk according to the absence or presence of the C9orf72 repeat expansion.

These aims are achieved through a family aggregation study.

The information generated by this family aggregation study will answer questions about the true rate of familial ALS, the risk of developing ALS in relatives of ALS patients with and without the C9orf72 repeat expansion, and will address the aggregation of neurodegenerative and neuropsychiatric conditions in relatives of ALS patients compared to controls. Findings from this study will direct future genetic studies in the field of ALS, and will also be clinically useful in the provision of information to relatives of ALS patients attending for genetic counselling.

2.3 Utilization of epidemiological information to identify subgroups for genetic analysis and characterise phenotypes

2.3.1 Description of ALS epidemiology in a population based register

To describe the epidemiology of ALS in the island of Ireland between 1996 and 2011, using the Irish ALS Register, and to use this information to inform the direction of future genetic studies.

Epidemiology of ALS in Ireland: Population-based registers are vital for the accurate reporting of disease epidemiology as well as allowing researchers to select patients for ongoing clinical trials, and epidemiological studies. As outlined in the previous chapter, the Irish ALS register, has been in existence since 1993, with complete ascertainment since 1996. A register in Northern Ireland has also been in existence since 2005.

Specific aims:

1. Analysis of the epidemiological and survival features of 1304 ALS patients on the Irish ALS register in the period 1996-2011

2. Comparison of the epidemiological and survival features of ALS patients from Ireland and Northern Ireland in the period 2005-2010
These aims were achieved through interrogation of informative data from the Irish ALS data set (1996-2011), and the Northern Irish ALS data set (2005-2010).

**Epidemiology of FALS:** Familial ALS cases have proven useful in gene discovery. A thorough study of the epidemiological features of familial ALS cases may direct future genetic work.

Specific aims:

1. To define the epidemiological features of familial ALS cases on the Irish ALS register, and to determine whether subgroups of familial cases exist that may be used to inform choices for genetic studies

These aims were achieved through interrogation of informative data from the Irish ALS data set (1996-2011), including demographic and survival information, cognitive data, geographic location at diagnosis, and patient surname.

### 2.3.2 Characterization of C9orf72 phenotype

To describe the clinical phenotype of ALS patients carrying the C9orf72 repeat expansion using epidemiologic data from the Irish ALS Register.

The recently discovered C9orf72 repeat expansion may be associated with half of all familial ALS cases. Scant data on the associated phenotype was reported in the seminal papers reporting the new gene.

Specific aims:

1. To use data from the Irish ALS Register to describe the phenotype of ALS in patients carrying the C9orf72 repeat expansion

2. To examine the cognitive phenotype associated with the C9orf72 repeat expansion in ALS patients

This aim achieved as a population-based longitudinal study of Irish ALS patients, started in 2006, is ongoing. Information available for analysis includes DNA, demographic and clinical information, survival outcome, neuropsychological data, neuroimaging characteristics, and detailed family history information.
Chapter 3 Familial ALS

Chapter four outlines two studies, which attempt to answer the following unresolved questions with regard to Familial ALS:

Is there consensus among clinicians' use of the term Familial ALS? (Section 3.1)

What is the rate of FALS in the general population? (Section 3.2)

3.1 Study A: To determine if there is consensus among clinicians on the definition of the term Familial Amyotrophic Lateral Sclerosis

3.1.1 Introduction

As discussed in chapter one it is not apparent from the literature on ALS a standard definition for the term familial ALS exists. Absence of a universally accepted definition for FALS could potentially lead to lack of clarity when including 'familial cases' in genetic studies, when giving genetic counselling advice to family members, as well as difficulty in the interpretation of epidemiological studies between countries, and also in the comparison of rates of genetic causes of FALS between research groups.

3.1.2 Aims

1. To determine whether a consensus for the term 'FALS' exists among clinicians

2. To propose criteria for Familial ALS

The aim of this study is to determine, using survey methodology, whether a consensus exists among clinicians regarding a standard definition for FALS, to examine how the term is applied to patients with ALS in clinical practice, and to seek opinion as to the need for a consensus meeting on the definition of FALS. Criteria are proposed for familial ALS.

3.1.3 Method

A questionnaire was devised (Appendix A). The questionnaire sought information in five domains:

A. Demographic information

B. Assessment of clinicians' opinion on the existence of a standard definition for FALS.
C. Assessment of the definition of FALS in use by the respondent in their clinical practice

D. Clinicians practice of classifying patients when confronted with a possible FALS pedigree

E. Assessment of the need for a consensus meeting to define FALS

An online version of the questionnaire was distributed using SurveyMonkey® to ALS research mailing lists in Europe (ENCALS), North America (NEALS), Australia, and India.

3.1.4 Results

A. Demographic information: There were 95 respondents from 15 countries (see table below). 61 (64.9%) of the respondents were male. 68 (75.6%) of the respondents were neurologists, and the remainder trainee neurologists, and clinical geneticists (21.1% and 4.4% respectively). 80 (85.1%) of respondents declared that they had a special interest in ALS.

<table>
<thead>
<tr>
<th>Country</th>
<th>Number respondents; number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ireland</td>
<td>16 (16.9%)</td>
</tr>
<tr>
<td>UK</td>
<td>7 (7.4%)</td>
</tr>
<tr>
<td>Germany</td>
<td>10 (10.5%)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>Poland</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>USA</td>
<td>35 (36.9%)</td>
</tr>
<tr>
<td>Australia</td>
<td>4 (4.2%)</td>
</tr>
<tr>
<td>France</td>
<td>3 (3.2%)</td>
</tr>
<tr>
<td>Italy</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Canada</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2 (2.1%)</td>
</tr>
<tr>
<td>Sweden</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>India</td>
<td>3 (3.2%)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>3 (3.2%)</td>
</tr>
<tr>
<td>No response</td>
<td>4 (4.2%)</td>
</tr>
</tbody>
</table>

Table 3.1 Questionnaire response breakdown by country of origin
B. Assessment of clinicians' opinion on the existence of a standard definition for FALS:
Respondents were asked if they thought that there was a standard definition among neurologists for FALS. One-third of total respondents were of the opinion that neurologists were using the same definition for FALS (33.3%, 30). There was a statistically significant difference when sub-group analysis based on country of practice was carried out; over half of respondents from North America (51.4%, 18) stated that there was a standard definition for FALS in use among neurologists compared to less than a quarter of respondents from Europe (22.0%, 9) [p= 0.015].

C. Assessment of the definition of FALS in use by the respondent in their clinical practice: Respondents were provided with five possible definitions for FALS. They were asked to select the definition that they believed to be most commonly used in the FALS literature. There was no consensus among the respondents (table 4.2).
Respondents were then provided with same five definitions and asked to select the one that most matched their own clinical practice (table 4.2). Again there was no consensus but the preferred definition was 'a patient with ALS with either a first or second degree relative also with ALS' (37.8%, 31). Respondents were also given the option of supplying their own definition if it differed from the five options provided.
Table 3.2 Breakdown of responses

In sub-group analysis, the preferred response for the definition of FALS among respondents who stated that they had a special interest in genetics and FALS, was 'a patient with ALS with any relative with ALS, no matter how distant' (34.8%, 8). Respondents were also given the opportunity to express their definition for FALS in their own words. Below are a number of examples:

'Family history of ALS or positive genetic test'

'None I do not believe in it (a definition). Anyone who has a family history that I obtain in clinic'

'In patients with a first degree relative I am more or less certain that they have familial ALS, if there is a second degree relative, I am very confident that there is familial ALS'
'As ALS is rare, the probability of two affected members in one family by chance, even if second degree is lower than variable penetrance.'

'Any family with two or more individuals with ALS connected by inheritance. Depending on the penetrance of a gene these individuals may be connected through non-penetrant carriers. The chance that these two individuals are sporadic cases can be determined statistically.'

'More than two patients (relatives) with common predecessor, no matter how distant horizontally and/or vertically located in the pedigree, with proven ALS diagnosis according to the internationally accepted criteria, with the same or different clinical type of the disease (age of onset, onset region, etc.), with or without proven mutation/s.'

'I tend to lump them all together. Some patients have an a pedigree that makes an autosomal dominant, highly penetrant heredity likely, and these are definitely FALS. Others have only a more distant relative with ALS, or even with another neurodegenerative disease. These later may share a susceptibility gene of some kind, rather than one of the known ALS genes, such as SOD1. Lastly, patients are occasionally discovered to carry an SOD mutation and have not other known family member with clinical ALS, and I also lump these into the FALS category.'

'I don't use the definition of FALS unless there is a known genetic alteration that has been shown to cause ALS or there is a genetic pattern of Mendelian transmission-either dominant, recessive, autosomal or X linked.'

D. Clinicians' practice of classifying patients when confronted with a possible FALS pedigree: Respondents were then provided with eight pedigrees and asked whether they would diagnose the kindred with FALS (see figure below).
If a patient presented to your clinic with the following family history, would you record them as having FALS?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
<th>Maybe</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>79.5% (70)</td>
<td>1.1% (1)</td>
<td>19.3% (17)</td>
</tr>
<tr>
<td>B</td>
<td>97.7% (86)</td>
<td>0% (0)</td>
<td>2.8% (2)</td>
</tr>
<tr>
<td>C</td>
<td>35.2% (31)</td>
<td>12.6% (12)</td>
<td>51.1% (45)</td>
</tr>
<tr>
<td>D</td>
<td>36.4% (32)</td>
<td>20.5% (18)</td>
<td>43.2% (38)</td>
</tr>
<tr>
<td>E</td>
<td>76.1% (67)</td>
<td>2.3% (2)</td>
<td>21.6% (19)</td>
</tr>
<tr>
<td>F</td>
<td>37.9% (32)</td>
<td>12.9% (11)</td>
<td>49.4% (42)</td>
</tr>
<tr>
<td>G</td>
<td>13.8% (12)</td>
<td>42.5% (37)</td>
<td>43.7% (38)</td>
</tr>
<tr>
<td>H</td>
<td>10.5% (9)</td>
<td>51.2% (44)</td>
<td>38.4% (33)</td>
</tr>
</tbody>
</table>

**Figure 3.1** Respondents were given the option to say if they would consider the following kindreds to have FALS or not.

In cases A) and B) the level of consensus was high. However, in over half of the pedigrees the majority of respondents chose the 'Maybe' option. 51 (58.6%) respondents gave answers that differed to the definition that they had previously stated that they used in clinical practice. 59 (67.0%) of respondents stated that they carried out genetic testing on patients who had a family history of ALS. Only nine (10.3%) respondents stated that they routinely carry out genetic testing on ALS patients with no family history. 52.3% of respondents (45) stated that they routinely refer patients with FALS for genetic counseling.

**E. Assessment of the need for a consensus meeting to define FALS:** 65 (74.7%) respondents agreed that a consensus meeting would be helpful in defining FALS.
3.1.5 Discussion

This study demonstrates that there is no standard definition for the term familial ALS. Although the majority of respondents agreed that having one other affected family member with ALS was sufficient to constitute a diagnosis of FALS, opinions differed as to whether this relative should be at the very least a first-degree relative, a second-degree relative or any relative, no matter how distant. Despite this discrepancy, over one-third of respondents believe that the term FALS carries a standard definition among specialists. This is surprising considering that when confronted with a series of eight kindred scenarios the majority of respondents chose the "maybe" option in four of the scenarios, indicating a lack of consensus as to what constitutes the familial form of the disease[204]. The greatest degree of uncertainty was observed in relation to scenarios where a distant relative was affected. Even when clinicians had clearly stated their preferred definition of FALS, over half of respondents were inconsistent in their definition when confronted with specific scenarios.

As is the case for all conditions in which there is both a familial and sporadic form, a clear and uniformly applied definition is important. There are large differences in the reported rates of FALS across different countries[47], as evidenced by the marked heterogeneity demonstrated in the results of the meta-analysis in the following section. While it is plausible that these differences are due to founder effect within any given population, it is also conceivable that these differences arise from inconsistent definitions for the term FALS. The same is true for reported rates of SOD1, which vary widely among different research cohorts[204]. Again this disparity may be due to a true difference in the rate of SOD1, for instance no cases of SOD1 have been identified among Irish ALS patients (chapter five), but it is also possible that these differences are a function of inconsistencies in classification.

A consensus definition of what constitutes familial disease is also important when selecting kindred's for linkage analysis and exome sequencing. Among respondents with an interest in ALS genetics and familial ALS, the most common definition for FALS was ‘any relative with ALS, no matter how distant’. As outlined above fewer affected people within a family who are distantly are less likely to yield a pathological gene in studies.
75% of respondents agreed that there should be a consensus meeting on the definition of FALS. Such a meeting should include both neurologists and geneticists. The ideal outcome from such a meeting would be a consensus definition which is clear, concise and clinically applicable. An additional requirement for research applications could be that clinicians record the total number of people within the kindred and the degree of relatedness of the other affected family member. At present it is not possible to test the proposed classification system using published FALS kindred's because few studies have provided details regarding kindred size and number of affected family members.

3.1.6 Proposing criteria for Familial ALS

Before considering criteria for familial ALS it is important to consider the role that chance plays in 'familial ALS'. Chance occurrence of ALS in two family members is entirely possible but no mention of this factor in estimating familial disease, in ALS or other neurodegenerative diseases, has been made in the literature. Estimates of chance co-occurrence of ALS within family members of a chosen proband may be calculated in a number of ways; firstly this could be calculated theoretically using the lifetime risk for ALS and the average number of first and second degree members in a kindred, and secondly by carrying out a family aggregation study of the family members of ALS probands and matched control probands to estimate the actual chance occurrence of ALS among relatives of the control probands.

Theoretical estimate of chance co-occurrence: Using information for lifetime adjusted individual risk of dying from ALS (1:350 males and 1:472 females) and average kindred size (figure 3.2) it is possible to calculate the probability of a second person within a kindred developing ALS by chance alone.
The average lifetime risk of developing ALS in the general population is 1/411 (1 in 350 in men and 1/472 in women) [95]. To establish the overall risk of having another affected family member, the following formula is used; \(1-(1- t)^n\), where \(t\) is individual risk for each relative and \(n\) is the number of family members in the kindred. The risk for each family member is essentially additive (e.g 1/411+1/411+...). This also depends on the age of affected members of the kindred as this lifetime risk assumes that the person has lived to old age. Given that the average number of adult first- and second-degree family members in an average kindred is 17 (http://www.cso.ie/en/index.html) the expected theoretical rate of any given person having another family member affected with ALS purely by chance, in Ireland, is 4.1% (17/411). To summarize, if people are selected at random from the population and asked about family history in a methodical way then 1-in-25 people will have a family history of ALS purely by chance.

The likelihood of an affected person having two affected relatives can also be estimated. The risk of two other family members being affected is multiplicative \([1-(1- t)^n ]x[1-(1- t)^n]\]. Figure 3.3 demonstrates that the likelihood of having two other family members affected by

Figure 3.2 Average family structure (green=first-degree relative, red=second-degree relative, black=affected individual)
chance is negligible and therefore one can conclude that families with more affected family members are better candidates for genetic studies.

![Graph showing the risk of having other family members affected by chance depending on kindred size where lifetime risk of ALS is 1/411](image)

Figure 3.3 The risk of having other family members affected by chance depending on kindred size where lifetime risk of ALS is 1/411

Research groups that study high proportions of kindreds with only two affected members are likely to observe a rate of genetic mutations that is lower than in groups which draw from kindreds with larger affected numbers, as larger families with more affected members are statistically less likely to occur by chance and more likely to have a genetic cause that is driving the familial aggregation of ALS.

In figure 3.4, the model was created by assuming that 50% of FALS cases occurred by chance in families where only two members are affected, and that less than 1% of FALS cases occurred by chance in families where three members are affected. This model assumes that SOD1 accounts for 10-20% of FALS cases and that the C9orf72 repeat expansion accounts for 40-60% of FALS cases, in all cases not occurring by chance e.g 50% in families where only two members are affected and 99% in families where three members are affected.
Figure 3.4 Percentage of SOD1 positive and C9orf72 positive results from a cohort depending on the proportion of 2- and 3- affected family member kindreds included

As the studies include families with more affected members (toward right side of the figure), where one assumes that chance plays a smaller role and heritable factors play a larger role, the percentage of genes found is higher.

After careful review of the literature, a consensus seeking questionnaire, and consideration of chance co-occurrence, I propose that a patient with a reported family history of ALS should be categorized into one of four groups: Definite, Probable, Possible and Suspected FALS. These are simple criteria based on an arbitrary number of affected family members that seek to clarify reports of familial ALS.
### Criteria for Familial Amyotrophic Lateral Sclerosis

<table>
<thead>
<tr>
<th>Definite FALS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History:</strong> A patient with <strong>two first or second degree relatives with ALS</strong></td>
</tr>
<tr>
<td><strong>Genetics:</strong> A patient with one relative with ALS, and testing positive for an ALS gene</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probable FALS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History:</strong> Patient with <strong>one first or second degree relative with ALS</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Possible FALS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History:</strong> Patient with one or more third degree relative with ALS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suspected FALS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>History:</strong> Patient with one or more affected relative with ALS of fourth degree or greater</td>
</tr>
</tbody>
</table>

**Note:**
1. Patients with a family history of FTD should be recorded and considered for C9orf72 testing.
2. Record the size of the kindred and the relationship between the affected individuals.

First degree: Parent, sibling, child. Second degree: Grandparent, grandchild, aunt/uncle, niece/nephew. Third degree: First cousin, great-uncle etc.

---

**Table 3.3 Proposed criteria for Familial ALS**

Families with three or more affected members, and families with two or more affected members where one of them tests positive for an ALS gene, should be categorized as Definite FALS. Kindreds with two affected first- or second-degree relatives, without a known gene, should be categorized as Probable FALS, and that kindreds with two or more third-degree relatives should be defined as Possible FALS. Patients with one affected distant family member (fourth-degree or more distant) should be considered as Suspected FALS.

Patients with ALS who do not have another affected family member but do have a family history of FTD should be considered for testing for the C9orf72 repeat expansion. Chapter six will discuss the finding that in an Irish cohort, a family history of FTD in an ALS patient is the strongest predictor for the presence of a C9orf72 repeat expansion, yielding an odds ratio of 102.2 (95% CI 12.3-845.5, p<0.0001) using logistic regression analysis of a population based cohort of 191 ALS patients.

**3.1.7 Conclusion**

Consistency in the definition of FALS will render epidemiological and genetic studies more transparent and homogenous, and will in turn permit more accurate comparison between
geographic regions. We also contend that further epidemiological studies using this categorization will allow better predictive models for genetic counselling purposes.

It is likely that similar inconsistencies apply to other conditions, such as Parkinson's disease and Alzheimer disease, in which both familial and sporadic disease occur. A consensus initiative to provide precise definitions of familial and sporadic disease is urgently required.
3.2 Study B: To report the rate of Familial Amyotrophic Lateral Sclerosis using systematic review and meta-analysis methodology

3.2.1 Introduction: rate of familial ALS

As discussed in chapter one, Kurland and colleagues[46] published a paper in 1955 which reported that the rate of FALS was 10%. This finding has been unquestioned in the literature ever since.

Population-based registries do record the rate of familial ALS reported by ALS patients, however as demonstrated in the previous section, the definition used for FALS between different populations may vary.

3.2.2 Aims

1. To determine the reported rate of familial ALS among published population-based studies.

The aim of this study is to perform a systematic review and meta-analysis of all studies that present original data reporting a rate of FALS (i.e. the proportion of familial cases amongst all ALS cases, either in a defined population or in a case series) to estimate the reported rate of FALS across studies. Analysis of the population-based frequency of FALS will be undertaken and, where possible, a geographic comparison will be made between this frequency and the frequency of known SOD1 mutations.

3.2.3 Methods

Systematic search: A Medline literature search was performed to identify all studies on FALS, in addition to studies reporting ALS incidence and prevalence published from 1966 to October 2009 (This meta-analysis was undertaken in October 2009). The MeSH terms "ALS", "Amyotrophic Lateral Sclerosis", "FALS", "Familial Amyotrophic Lateral Sclerosis", "familial motor neuron(e) disease", "motor neuron(e) disease", "MND", "incidence", "prevalence" and "mortality" were used. Additional references were identified from cited articles. Where no information was reported on the rate of FALS in a population-based study, the corresponding
author was contacted where possible. Unpublished up-to-date data from the Irish ALS prospective population based register was also used.

**Eligibility criteria and Data Collection:** All studies presenting original data that reported a rate of FALS (i.e. the proportion of familial cases within a defined cohort) were included in the systematic review. Only studies that demonstrated complete enrollment, either in the form of population-based registry or in sequential case series, were analyzed. Studies with non-random enrollment or cohorts enriched for familial disease were not included in analysis. Studies fulfilling inclusion criteria were grouped together according to the type of data presented; 1) Prospective registry-based studies that aim to capture all cases within a given geographic region in order to define incidence and prevalence, 2) Retrospective studies that attempt to capture all cases in a given geographic region with the aim of estimating incidence and prevalence, 3) Prospective cases series, 4) Retrospective case series.

**Statistical analysis:** In each study, the rate of FALS was defined as the number of reports of familial ALS among all cases of ALS. Statistical analysis was carried out to combine proportions using the meta-function in R [205]. The inverse variance method was used to pool proportions. Both fixed and random effects models were estimated and confidence intervals of 95% were calculated. The following code in R was used for calculating the pooled results:

prospcas<-c(2,53,59,31,9,6,53,52)
prospmnn<-c(130,1260,1226,472,174,143,1170,708)
mymeta<-metaprop(prospcas,prospmnn)
retrospcas<-c(10,3,4,2,6,5,6,3,4,2,1,1,5,7)
retrospn<-c(244,77,143,36,139,43,128,65,182,133,58,84,186,148)
mymetar<-metaprop(retrospcas,retrospn)
retrocs<-c(7,27,23,5,3,12,3,20)
retrocn<-c(251,580,531,58,52,140,318,307)
mymetar2<-metaprop(retrocs,retrocn)
prospcs<-c(114,33,4)
prospcn<-c(1200,668,167)

mymetap2<-metaprop(prospcs,prospcn)

first2<-c(prospcas,retrospcas)

first2n<-c(prospmn,retrospn)

mymetaf2<-metaprop(first2,first2n)

summary(mymetaf2)

last2<-c(retrocs,prospcs)

last2n<-c(retrocn,prospcn)

mymetal2<-metaprop(last2,last2n)

summary(mymetal2)

totc<-c(first2,last2)

totn<-c(first2n,last2n)

mymetat<-metaprop(totc,totn,c("Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a","Study b","","Study a"))

summary(mymetat)

#totc2<-c(first2,retrocs,114)

#totn2<-c(first2n,retrocn,1200)

#mymetat2<-metaprop(totc2,totn2)

#summary(mymetat2)

sink("out20100212.txt")

df("out20100212.pdf")

summary(mymeta)

summary(mymeta)

summary(mymetar)

summary(mymetar2)

summary(mymetap2)

summary(mymetaf2)

summary(mymetal2)
Systematic review: Fifty-four epidemiologic studies provided original information on cohorts of patients with ALS [46, 50, 54-108]. A rate of FALS was reported in 38 of these studies though this was not the primary objective in any of them. Five studies were not included in analysis [54, 55, 105-107]. Of these, two were undertaken to genotype mutations in the ALS population, and both studies suffered from enrollment bias [54, 55]. Three studies reported data on more than one occasion [105-107] and therefore only the most recent study was included in the meta-analysis. Reports from geographical areas with high-incidence clusters (e.g. Guam and the Kii Peninsula of Japan) were excluded [108].

Of the thirty-three studies analysed, 8 reported incidence data from prospective population-based registers; 14 reported retrospective incidence and prevalence data; 3 reported data from prospective case series of disease progression, and 8 reported data from retrospective case series. Only two studies stated how they defined FALS [73, 86]. In total 575 cases of FALS were recorded among 11,221 reported cases of ALS.

The type of study, rate of FALS, source of case ascertainment and case inclusion criteria are outlined in table 3.4.
<table>
<thead>
<tr>
<th>Type study</th>
<th>Country</th>
<th>Reference</th>
<th>Years</th>
<th>ALS cases</th>
<th>% FALS cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPB</td>
<td>Puglia, Southern Italy</td>
<td>56</td>
<td>1998-1999</td>
<td>130</td>
<td>1.5%</td>
</tr>
<tr>
<td>PPB</td>
<td>Piemonte, North Italy</td>
<td>57</td>
<td>1995-2004</td>
<td>1260</td>
<td>4.2%</td>
</tr>
<tr>
<td>PPB</td>
<td>Scotland</td>
<td>58</td>
<td>1989-1998</td>
<td>1226</td>
<td>4.8%</td>
</tr>
<tr>
<td>PPB</td>
<td>South-East England</td>
<td>59</td>
<td>2002-2006</td>
<td>472</td>
<td>6.6%</td>
</tr>
<tr>
<td>PPB</td>
<td>Washington, USA</td>
<td>60</td>
<td>1990-1994</td>
<td>174</td>
<td>5.2%</td>
</tr>
<tr>
<td>PPB</td>
<td>San Francisco, USA</td>
<td>62</td>
<td>1970-1986</td>
<td>708</td>
<td>7.3%</td>
</tr>
<tr>
<td>PPB</td>
<td>Uruguay</td>
<td>63</td>
<td>2002-2003</td>
<td>143</td>
<td>4.2%</td>
</tr>
<tr>
<td>PPB</td>
<td>Ireland Register data</td>
<td></td>
<td>1995-2009</td>
<td>1170</td>
<td>4.5%</td>
</tr>
<tr>
<td>RPB</td>
<td>New Zealand</td>
<td>65</td>
<td>1985-2006</td>
<td>244</td>
<td>4.1%</td>
</tr>
<tr>
<td>RPB</td>
<td>Rochester, USA</td>
<td>66</td>
<td>1925-1998</td>
<td>77</td>
<td>3.9%</td>
</tr>
<tr>
<td>RPB</td>
<td>Modena, Italy</td>
<td>67</td>
<td>1990-1999</td>
<td>143</td>
<td>2.8%</td>
</tr>
<tr>
<td>RPB</td>
<td>Jefferson, USA</td>
<td>68</td>
<td>1998-2002</td>
<td>36</td>
<td>5.5%</td>
</tr>
<tr>
<td>RPB</td>
<td>Ontario, Canada</td>
<td>69</td>
<td>1978-1982</td>
<td>139</td>
<td>4.3%</td>
</tr>
<tr>
<td>RPB</td>
<td>Middle Finland</td>
<td>70</td>
<td>1976-1981</td>
<td>43</td>
<td>11.6%</td>
</tr>
<tr>
<td>RPB</td>
<td>Northern Sweden</td>
<td>71</td>
<td>1969-1980</td>
<td>128</td>
<td>4.7%</td>
</tr>
<tr>
<td>RPB</td>
<td>Cantabria, Spain</td>
<td>72</td>
<td>1974-1985</td>
<td>65</td>
<td>4.6%</td>
</tr>
<tr>
<td>RPB</td>
<td>Sardinia, Italy</td>
<td>73</td>
<td>1957-1980</td>
<td>182</td>
<td>2.2%</td>
</tr>
<tr>
<td>RPB</td>
<td>South-West Greece</td>
<td>74</td>
<td>1990-2003</td>
<td>133</td>
<td>1.5%</td>
</tr>
<tr>
<td>RPB</td>
<td>Belgrade, Yugoslavia</td>
<td>75</td>
<td>1985-1991</td>
<td>58</td>
<td>1.7%</td>
</tr>
<tr>
<td>RPB</td>
<td>Hong Kong</td>
<td>76</td>
<td>1989-1992</td>
<td>84</td>
<td>1.2%</td>
</tr>
<tr>
<td>RPB</td>
<td>Northern Denmark</td>
<td>77</td>
<td>1974-1986</td>
<td>186</td>
<td>2.7%</td>
</tr>
<tr>
<td>RPB</td>
<td>Hordaland, Norway</td>
<td>78</td>
<td>1970-1990</td>
<td>148</td>
<td>4.7%</td>
</tr>
<tr>
<td>PCS</td>
<td>Texas, USA</td>
<td>86</td>
<td>1982-1994</td>
<td>1200</td>
<td>9.5%</td>
</tr>
<tr>
<td>PCS</td>
<td>New York, USA</td>
<td>87</td>
<td>1973-1977</td>
<td>668</td>
<td>4.9%</td>
</tr>
<tr>
<td>PCS</td>
<td>Colorado, USA</td>
<td>88</td>
<td>1989-1991</td>
<td>167</td>
<td>2.4%</td>
</tr>
<tr>
<td>RCS</td>
<td>Brazil</td>
<td>79</td>
<td>1977-2004</td>
<td>251</td>
<td>2.8%</td>
</tr>
<tr>
<td>RCS</td>
<td>London, UK</td>
<td>80</td>
<td>1965-1984</td>
<td>580</td>
<td>4.7%</td>
</tr>
<tr>
<td>RCS</td>
<td>Rochester, USA</td>
<td>99</td>
<td>Pre 1955</td>
<td>58</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

Table 3.4 Studies included in meta-analysis

PPB = Prospective population based study
RPB = Retrospective population based study
PCS = Prospective case series
RCS = Retrospective case series
**Meta-analysis:** Pooled analysis of all studies generated a rate of FALS of 4.6% (95% CI 3.9-5.5%).

<table>
<thead>
<tr>
<th>Study type</th>
<th>Number of studies</th>
<th>FALS cases</th>
<th>Total ALS cases</th>
<th>Fixed effects Proportion</th>
<th>Random effects Proportion</th>
<th>I²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: Prospective population based registry</td>
<td>8</td>
<td>256</td>
<td>5,283</td>
<td>5.0% (4.4-5.6)</td>
<td>5.1% (4.1-6.1%)</td>
<td>56%</td>
</tr>
<tr>
<td>Group 2: Retrospective population based registry</td>
<td>14</td>
<td>59</td>
<td>1,666</td>
<td>3.7% (2.9-4.7)</td>
<td>3.7% (2.9-4.7)</td>
<td>0%</td>
</tr>
<tr>
<td>Group 3: Prospective case series</td>
<td>3</td>
<td>151</td>
<td>2,035</td>
<td>7.3% (6.2-8.4)</td>
<td>5.7% (2.5-9.9)</td>
<td>91%</td>
</tr>
<tr>
<td>Group 4: Retrospective case series</td>
<td>8</td>
<td>100</td>
<td>2,237</td>
<td>4.4% (3.6-5.2)</td>
<td>4.8% (3.8-5.3)</td>
<td>74%</td>
</tr>
<tr>
<td>Pooled results for all studies (Groups 1-4)</td>
<td>33</td>
<td>575</td>
<td>11,221</td>
<td>5.1% (4.7-5.5)</td>
<td>4.6% (3.9-5.5)</td>
<td>69%</td>
</tr>
</tbody>
</table>

Table 3.5 Meta-analysis results: Subgroup analysis and pooled analysis.

A degree of heterogeneity was noted between reported rates of FALS in the prospective population based registers available for analysis (I²=56%). The pooled result for rate of FALS for these studies was 5.1% (95% CI 4.1-6.1).

In the 14 retrospective population based studies, the pooled result for rate of FALS was 3.7% (95% CI 2.9-4.7).
Figure 3.5 Forrest plot of the prospective and retrospective population based studies

The individual rate of FALS for each country in Europe was plotted on a map to determine a possible geographic pattern (figure 3.6).

Figure 3.6 Geographic distribution in Europe of rates of FALS
Regions of relatively homogenous genetic origin, such as Ireland and Scotland, (both of which both have large prospective databases of over twelve hundred patients) exhibit almost the same rate of FALS. Lower rates are reported in the more diverse populations of southern Italy and the Balkan Peninsula. There was no a geographic correlation between FALS rates and reported SOD1 mutation rates for the same region.

3.2.5 Discussion

This analysis indicates that the commonly accepted frequency of 10% may represent an overestimation of the rate of FALS reported by patients with ALS. The population-based frequency of 5.1% is likely to represent the most accurate estimation of the reported rate of FALS, as population-based studies include all patients in a defined geographic area in a given period of time. Accordingly, reported rates of FALS of 10% from previously reported retrospective single centres are likely to have been biased by ascertainment from populations enriched by FALS cases.

This paper describes a meta-analysis of the reported rate of FALS in a number of epidemiological studies. It is important to make the distinction between the 'reported rate' of FALS and the 'true rate' of FALS. The reported rate of FALS in these studies is the best estimate of the rate of FALS reported to treating physicians among a population. None of these studies were carried out with the primary end-point of discovering the true rate of FALS.

Accurate reporting of the 'true rate' of FALS within a cohort is dependent on the availability of a detailed family history from every patient diagnosed with ALS, including all first- and second-degree relatives. If the patient is unaware of the family history it is helpful if they can nominate a relative with a better knowledge of the family members.

Geographic variation in the rate of FALS in Europe may reflect true population-based differences across different regions. Heterogeneity of the genetic substructure of European populations has been demonstrated recently[206], and these geographic differences may occur because of variability in the underlying genetic structure of the European population.
Difficulties in case ascertainment could also account for this difference. Evidence for between-population differences is also emerging for *SOD1*: a founder effect for the A4V mutation in *SOD1* has been recently identified in the USA, and this mutation is rare in Europe. Mutations in *OPTN* seem to be primarily prevalent in Japanese populations[140]. The existence of geographic variability for *TARDP* and *FUS* has yet to be established. The increased rate of FALS was higher in Finland (11.6%), and this may be due to a C9orf72 repeat expansion founder effect, which is believed to have arisen in Finland[116]. As more causative genes are identified in FALS, detailed analysis of the frequency of various mutations within individual FALS populations will become available.

In order to accurately record the true rate of FALS in a population-based cohort a prospective controlled study is required (see family aggregation study in chapter six). The aim of such a study would be to contact all population-based incident cases or a representative proportion of incident ALS cases over a subsequent number of years, to gather detailed family history information and use genealogical methods to identify all familial cases of ALS. Such a study would require an in-depth questionnaire to identify other cases of FALS within a family. Verification of reported cases would be required by physical examination where possible, medical records and death certificates. Death certification is a useful method of acquiring information on deceased members from previous generations. A grandparent is more closely related to the proband than a first cousin, but medical information may be more readily available on the latter. In order to get an accurate rate for FALS it is important to thoroughly research the health status of all first- and second-degree relatives.

Such a study would be required to routinely update the current records of all ALS cases because those defined as having sporadic ALS may subsequently have a family member diagnosed with ALS. Given that half of all familial cases of ALS have only two affected members within a kindred[48], it is reasonable to suppose that half of these patients are the first to be affected within their kindred and therefore appear to be sporadic. This effectively means that at any point in time, the reported rate of FALS is only 75% of the actual rate.
A study carried out in this fashion would give the best possible estimate of the absolute rate of FALS in a given population, however to get the true rate of FALS it is necessary to calculate the rate of cases of ALS that co-occur in families by chance and subtract this from the absolute rate.

The meta-analysis is limited by the absence of a clear definition of FALS in published studies. Only 6% (2/33) of studies included in the meta-analysis provided a definition for FALS. When recording family history status, the degree or relatedness and the size of the extended kindred are rarely considered, and a consensus seeking questionnaire determined that there is no standard definition for the term FALS among neurologists.

3.2.6 Conclusion

The reported rate of FALS across population-based studies rarely exceeds 5% of all cases of ALS. This contrasts with the generally accepted figure of 10%, which originates from a paper written in 1955[46].

Careful prospective population based analysis of kindreds using validated criteria for diagnosis of FALS is required to confirm that 5% represents the true population-based rate of familial ALS, and to determine whether the documented geographic variation in rates of FALS is confirmed.

<table>
<thead>
<tr>
<th>Accepted rate of FALS</th>
<th>Reported rate of FALS (meta-analysis)</th>
<th>Rate of FALS predicted to occur by chance</th>
<th>Rate of FALS in family aggregation study</th>
<th>Rate of family history of ALS in healthy controls</th>
<th>True rate of FALS (actual rate minus background rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>5.1%</td>
<td>4.1%</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Chapter 4 Epidemiology of ALS

4.1 General introduction

A complete and well-maintained population-based disease registry is crucial for researchers who wish to investigate the population-based epidemiology of disease. Registers are useful in determining the incidence rate and survival outcomes in a particular disease over time. Information generated by the register can aid in the discovery of disease subgroups, which may help genetic epidemiologists in their search for genes associated with the disease.

4.2 Study A: Epidemiology of ALS in the island of Ireland; 1996-2011

4.2.1 Description of the Irish ALS Register

The Irish ALS Register was set-up by Professor Orla Hardiman in 1993 and benefits from complete case ascertainment since 1996. The Republic of Ireland covers 70,283 square kilometres, and is divided into 26 geographic regions (counties). All people over the age of fifteen years who have been diagnosed with ALS, and are resident in the Republic of Ireland for longer than three years, are included on the register. A specialist research nurse identifies ALS cases by contacting all neurologists and geriatricians in the Republic of Ireland, reviewing HIPE (Hospital Inpatient Enquiry Scheme) computerized hospital discharge records, through the Motor Neuron Disease Association (a patient-support organization), and via patient self-registration. Patients give consent for their information to be stored on the Register. The Central Statistics Office provides information on all people who have MND or ALS recorded as primary, secondary or tertiary causes of death on their death certificates.

For inclusion on the register, extensive confirmatory measures such as clinical examination by a specialist, direct chart review, and assessment by neurophysiologist, is required. Clinical progression is tracked by regular telephone contact between the register coordinator, health care professionals, patients and carers, and by home visits by members of the ALS Research Group. To date, over 1,500 ALS cases are recorded on the register. An associated bank of
DNA samples has been in operation since 1999 and over eight hundred samples are stored for research purposes.

**Longitudinal study of cognition in ALS patients:** Incident ALS patients diagnosed from November 2006 to present, selected through the Irish ALS register, have been enrolled in a prospective longitudinal study of cognitive and behavioural function. Detailed longitudinal clinical, neurocognitive and behavioural data, structural magnetic resonance (MR) imaging, and survival data have been collected on this cohort, and DNA has been banked for genomic analysis. Cognitive and behavioural testing is performed at six-month intervals in patients' homes from inclusion in the study until they decline to participate or die. Exclusion criteria for the study include a history of traumatic brain injury, learning disability, alcohol dependence, and type 1 or uncontrolled type 2 diabetes mellitus. The neuropsychological battery evaluated multiple cognitive domains including executive function, memory function, language, visiospatial domains and, behaviour. Matched controls are recruited for comparative purposes. Based on corrected scores patients are categorised as "Cognitively normal" (cognitive or behavioural), "ALS-FTD" (fulfilling the Neary criteria for FTD), "Executive dysfunction" (patients scoring two standard deviations below the mean of healthy controls on at least two executive tasks), and "Non-executive impairment/NECI" (patients with visuo-spatial, language or memory impairments).

**4.2.2 Aims**

1. Analysis of the epidemiological and survival features of 1304 ALS patients on the Irish ALS register in the period 1996-2011
2. Comparison of the epidemiological and survival features of ALS patients from Ireland and Northern Ireland in the period 2005-2010
3. To define the epidemiological features of familial ALS cases on the Irish ALS register including geographic location, and surname frequency, and to determine whether subgroups of familial cases exist that may be used to inform choices for genetic studies
4.2.3 Methods

Database preparation: An SPSS database was created from the Irish ALS register documenting demographic information on all cases of definite, probable and possible ALS cases diagnosed in the Republic of Ireland between 1st January 1996 and 31st December 2011. Variables recorded included; sex, date of birth, county of residence, address, surname, date of birth, date of first symptom onset, site of onset (bulbar/limb/respiratory), date of diagnosis, El Escorial category at diagnosis, self reported family history of ALS (any affected family member), attendance at Beaumont hospital specialist ALS clinic, use of riluzole, use of non-invasive ventilation, use of gastrostomy, date of gastrostomy insertion, and date of death or censor. An attempt was made to contact any patient with a survival time greater than four years from diagnosis, to ensure that they were still living.

Data entry was then checked manually and missing variables were updated where clinical information was available. A number of variables were created including 'age at onset', 'age at diagnosis', 'time from onset to diagnosis', 'age category at diagnosis' (20-39, 40-49, 50-59, 60-69, 70-79, 80+), and 'time period of diagnosis' (1996-1999, 2000-2003, 2004-2007, 2008-2011). The 'time period of diagnosis' intervals of four years were chosen as an arbitrary time interval, as sixteen years of data were available for analysis.

Correction for underlying population structure: Incidence rates reported for time periods and age categories were manually corrected using the direct method to reflect the underlying population structure at the mid point of each time period. Population estimates were available from Central Statistics Office, the national body responsible for conducting the Irish population census every four years.


Interrogation to identify subgroups within the familial ALS cohort: A number of factors are assessed for evidence of subgroups within the cohort of familial patients; the frequency of age at diagnosis is assessed to look for evidence of bimodal age at onset, the frequency of
survival length in familial patients is also assessed; cognitive outcomes in familial patients are compared.

**Application of geo-coordinates and mapping:** To assess visually if there was clustering of familial cases in any particular region a web-based health resource, provided by the Health Service Executive (HSE), called Health Atlas Ireland was used. All addresses from the Republic of Ireland were converted to an x,y coordinate (‘geocode’) by Health Atlas Ireland technology, and mapped onto the island of Ireland. This application does not correct for underlying population structure, and only seeks to provide a visual overview of case location on the island of Ireland.

The proportion of familial ALS cases compared to all reported ALS cases for each geographic county is reported (26 in total) to determine whether any geographic counties have a disproportionate amount of familial case. As proportion is being compared for a geographic region, correction for the underlying population is not necessary. The mean proportion of FALS is then calculated, and comparisons are made on a county-by-county basis to identify counties that have a higher than statistically expected number of familial cases (2 or more standard deviations above the mean proportion).

**Surnames:** To identify any surname that may appear more frequently than would be expected by chance among male ALS patients on the register (n=785), a comparison was made between the frequency of the ten most common Irish names from the population census. Two sources are available on the frequency of surnames in the Republic of Ireland (Matheson - Special Report on Surnames in Ireland [1981], and Murphy - Studies in Irish Genealogy and Heraldry [2009]). While surnames in Ireland are region specific, the population frequency of the surnames comes from the census, which records surnames of the 4,000,000 inhabitants of Ireland from all geographic areas. Likewise the ALS register is population-based and records ALS cases from all geographic regions of Ireland without bias, therefore the two are directly comparable. The frequency proportion from the ALS registry is reported with a 95% confidence interval calculated from proportional count data.
Comparisons are made to see if any surnames appear significantly more on the register than would be expected by chance.

**Statistical analysis:** Demographic and clinical characteristics of the participants are reported as percentages for categorical variables and means/medians for continuous variables. Demographic information was tested for normality by plotting histograms and using the K-S test for normality. Comparisons were made using Chi-square or Fisher Exact test or 2-sample t-test as appropriate. Where variables are normally distributed a mean is reported. Where variables are not normally distributed a median is reported and non-parametric tests are used. Univariate analysis of covariance (ANCOVA) was carried out to adjust for confounding factors where appropriate. Spearman’s Rho test was used to assess the degree of correlation strength between variables with a non-parametric distribution. Survival analysis: Survival time was defined as time from diagnosis to death, co-varied for time from onset to diagnosis. Time from symptom onset to diagnosis was compared between groups to ensure lack of lead-time bias. Patients were followed up from their time of entry onto the register until death or censor date (31st December 2011). Patients who were alive at the time of analysis were censored.

Dates for prevalence ascertainment were set at 31st December 2003, and 31st December 2010.

To ensure that period of diagnosis did not affect cohort outcome, a separate survival analysis was carried out looking at four-year survival time between the four time period groups. The censor dates were set as follows: 1996-1999 – 31st December 1999; 2000-2003 – 31st December 2003; 2004-2007 – 31st December 2007; 2008-2011 – 31st December 2011.

In all survival analysis univariate assessment of the survival effect of categorical variables was carried out using Kaplan-Meier (KM) survival analysis and equality of outcome was assessed using the log-rank test. Cox proportional hazards method was used for multivariate survival analysis using backward elimination (Wald test), estimation of hazard ratio (HR), and 95% confidence intervals. Multivariate survival analysis adjusted for time from onset to
diagnosis, age at onset, site of onset, sex, attendance at Beaumont clinic, family history, use of riluzole, use of gastrostomy, use of non-invasive ventilation (NIV), and time period. All tests were 2-tailed and statistical significance was set at p<0.05. Bonferroni correction was made for multiple comparisons. Statistical analysis was carried out using SPSS version 18 (SPSS Inc, Chicago, IL).

**Presentation of results:** Results are presented for the time period 1996-2011, and also in four-year time periods (1996-1999, 2000-2003, 2004-2007, 2008-2011). Results are presented for all ALS cases, and, where possible, for sporadic cases versus familial cases.

**Ethical approval:** Ethical approval for this study was granted by the local ethics board at Beaumont Hospital, Dublin 9, Ireland.
4.2.4 Results: Capture-Recapture

Two independent sources were used to estimate accuracy of the Irish ALS register; source A - cases self reported, reported by neurologists, and recorded on HIPE (2002-2006), and source B: ALS as a reported cause of death on death certificates (2002-2006), were used in capture-recapture calculations. Source A had unique 60 cases, and source B had 79 unique sources, while 318 cases were reported by both sources[207]. Using the capture-recapture formula provided in section 1.2.6, this gives a capture-recapture estimate of 15 unobserved patients in the five-year period. This gives a coverage rate of 96% for the ALS register in Ireland.

4.2.5 Results: General demographics

1304 people from the Republic of Ireland were diagnosed with definite, probable or possible ALS in the sixteen-year period from 1st January 1996 to 31st December 2011.

<table>
<thead>
<tr>
<th>Year Period</th>
<th>Total Cases</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996-2011</td>
<td>1304 (100%)</td>
<td>297 (100%)</td>
<td>324 (100%)</td>
<td>317 (100%)</td>
<td>366 (100%)</td>
</tr>
<tr>
<td>1996-1999</td>
<td>1220 (93.6%)</td>
<td>285 (96%)</td>
<td>310 (95.7%)</td>
<td>289 (91.2%)</td>
<td>336 (91.8%)</td>
</tr>
<tr>
<td>2000-2003</td>
<td>84 (6.4%)</td>
<td>12 (4%)</td>
<td>14 (4.3%)</td>
<td>28 (8.8%)</td>
<td>30 (8.2%)</td>
</tr>
</tbody>
</table>

Table 4.1 Number of cases diagnosed in each four-year period. Proportion of sporadic (SALS) and familial (FALS) cases reported in each period

The rate of familial ALS recorded on the Irish ALS register differed across the different time periods, with higher rates in the more recent time periods (p=0.006).

4.2.6 Results: Site of onset and gender distribution

Over the period 1996-2011 the ratio of males to females diagnosed with ALS was 1.3:1.0.

The site of onset remained constant over the time periods.
Table 4.2 Sex and site of onset broken down by sex and time period

The proportion of males to females diagnosed was the same in sporadic cases as it was in known familial cases; 56.7% (692) of sporadic cases were male compared to 56% (46) of familial ALS cases (p=0.891). There was no difference between the sites of onset of sporadic cases compared with familial cases; 58.1% (696) of sporadic cases had limb onset compared to 60.9% (52) of familial cases (p=0.475).

### 4.2.7 Results: Age at symptom onset and diagnosis of ALS

The age at symptom onset and disease diagnosis was compared between period of diagnosis, sex, and family history.
Table 4.3 The histogram depicts the frequency of age of onset in years (x-axis) for all cases (y-axis) diagnosed between 1996-2011. Table demonstrates the age at onset and diagnosis for the period of diagnosis, sex, and family history. Standard deviation is reported in brackets.

Age of onset and age of diagnosis of disease was significantly lower in familial cases than sporadic cases (onset p=0.007, diagnosis p=0.003). There was no difference in the time from symptoms onset to diagnosis between the four time periods (p=0.12).

The mean ages at symptom onset and age at diagnosis for all cases were plotted for each time period and demonstrate that there is a trend towards an increase in age at symptom onset and diagnosis over time.
Figure 4.1 Demonstrate the increase in age at onset and diagnosis over time
(Difference across groups for age of onset p=0.11; difference across groups for age of diagnosis p=0.24)

4.2.8 Results: Proportion of cases diagnosed in each age category
The proportion of all patients people diagnosed in each time period is shown in figure 4.2.
The proportions are constant across the time periods (p=0.246).
Figure 4.2 Proportion diagnoses made in each age categories for all time periods.

Significantly more familial cases were diagnosed in the younger age categories than sporadic cases (p=0.005). This is demonstrated in figure 4.3.

Figure 4.3 Proportion of cases diagnosed in each age category for SALS and FALS.
4.2.9 Results: Incidence

There was no difference in the corrected incidence rate over the different time periods.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>All ALS cases</td>
<td>1304</td>
<td>297</td>
<td>324</td>
<td>317</td>
<td>366</td>
</tr>
<tr>
<td>Midpoint Population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>estimate &gt;15 years</td>
<td>3,195,004</td>
<td>2,866,600</td>
<td>3,089,333</td>
<td>3,299,168</td>
<td>3,462,185</td>
</tr>
<tr>
<td>Incidence rate per 100,000</td>
<td>2.6</td>
<td>2.6</td>
<td>2.6</td>
<td>2.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Table 4.4 Incidence rates of ALS for different time cohorts

The corrected incidence rates were plotted for each age category and for each time period, and the results were plotted.

Figure 4.4 Corrected incidence rate plotted for each time period and age category
4.2.10 Results: Prevalence

There was no difference in prevalence between the two time periods.

<table>
<thead>
<tr>
<th></th>
<th>Ireland 2003</th>
<th>Ireland 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>200 cases</td>
<td>237 cases</td>
</tr>
<tr>
<td>Population &gt; 15 years old</td>
<td>3,145,400</td>
<td>3,311,500</td>
</tr>
<tr>
<td>Population &gt; 15 years old</td>
<td>6.4 per 100,000 (95% CI 5.5-7.2)</td>
<td>6.8 per 100,000 (95% CI 5.9-7.6)</td>
</tr>
</tbody>
</table>

Table 4.5 Comparison of ALS prevalence

4.2.11 Results: Use of intervention

The rate of intervention use was compared between the different time periods. The table demonstrates the rate of intervention usage over the four time periods. There is a significant difference in the rate of usage of each intervention over the time periods (p<0.0001). Over the four time periods the interval between symptom onset to gastrostomy tube insertion has decreased from 53 months (SD 35.4) in the period 1996-1999, to 16.2 months (SD 8.3) in the most recent time period, 2008-2011.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Riluzole</td>
<td>71.1% (905)</td>
<td>42.8% (127)</td>
<td>74.4% (241)</td>
<td>80.4% (255)</td>
<td>84.4% (282)</td>
</tr>
<tr>
<td>NIV</td>
<td>23.9% (289)</td>
<td>5.1% (15)</td>
<td>23.8% (77)</td>
<td>32.6% (103)</td>
<td>34.4% (94)</td>
</tr>
<tr>
<td>Gastrostomy</td>
<td>26.6% (321)</td>
<td>14.1% (42)</td>
<td>36.7% (119)</td>
<td>27.1 (86)</td>
<td>27.3% (74)</td>
</tr>
<tr>
<td>All cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attended specialist ALS clinic in Beaumont</td>
<td>63.3% (761)</td>
<td>66.6% (195)</td>
<td>55% (177)</td>
<td>61.6% (194)</td>
<td>71.4% (195)</td>
</tr>
</tbody>
</table>

Table 4.6 Use of interventions in ALS over the four time periods, and attendance at the specialist ALS clinic in Beaumont Hospital
Familial cases of ALS were prescribed more riluzole (83.3% versus 70.3%, p=0.011), were
given access to NIV more frequently (28.9% versus 23.6%, p<0.0001), and had more
gastrostomy tubes inserted (36.8% versus 25.9%, p=0.036) than sporadic ALS cases.
There is an increase in the rate of attendance of ALS patients at the specialist ALS clinic in
Beaumont Hospital in the most recent time period (p<0.0001).

4.2.12 Results: Survival in ALS between time cohorts

The mean survival time from symptom onset to death in Irish ALS patients diagnosed
between 1996 and 2011 was 46 months (95% CI 41.4-50), and the median survival from
symptoms onset to death was 28 months (95% CI 26.6-29.4). The mean survival time from
diagnosis to death in Irish ALS patients diagnosed between 1996 and 2011 was 27 months
(95% CI 24.9-29.7), and the median survival was 15 months (95% CI 14-16).

Age matched univariate analysis revealed no difference in survival time from diagnosis to
death in patients diagnosed in different time periods (p=0.48). Multivariate analysis,
adjusting for time from onset to diagnosis, age at onset, site of onset, sex, attendance at
Beaumont clinic, family history, use of riluzole, use of gastrostomy, use of non-invasive
ventilation (NIV), and time period, failed to demonstrate any survival advantage for diagnosis
in one time period over another. Survival duration was calculated from date of diagnosis
(inclusion on register) to date of death or end of study censor date (31st December 2011).

Factors associated with shorter survival from disease diagnosis to death on multivariate
analysis included; older age at onset (HR 1.03; 95% CI 1.02-1.04; p<0.0001), and shorter
interval from disease onset to diagnosis (HR 1.01; 95% CI 1.01-1.02; p=0.0001).
A second survival analysis was carried out to look for period effects; follow up was from patient entry into a time period to death or the end of that four year time period. There was no significant difference between survival length seen between patients in different time periods on univariate or multivariate analysis (p=0.541).

4.2.13 Results: Interventions and attendance at specialist clinics

Factors associated with an increase in survival from disease diagnosis to death on multivariate analysis included the use of riluzole (HR 0.7; 95% CI 0.6-0.8; p<0.0001) and attendance at the Beaumont ALS clinic (HR 0.7; 95% CI 0.6-0.8; p<0.0001).

Figure 4.5 Multivariate model of survival from diagnosis in different time periods (p=0.48)
Figure 4.6 Attendance at the Beaumont ALS clinic was associated with a median survival time of 18 months from diagnosis of disease (95% CI 16.6-19.4), compared to a median survival of 10 months (95% CI 8.6-11.4) in those not attending the clinic (p<0.0001).

The use of non-invasive ventilation (HR 1.4; 95% CI 1.2-1.7; p<0.0001) was associated with a shorter survival.

4.2.14 Results: Survival in sporadic onset versus familial onset ALS cases

Univariate analysis showed a significant increase in survival time for patients with familial disease compared with sporadic disease (p=0.002, see figure 4.7).
Figure 4.7 Univariate comparison of survival in familial and sporadic ALS cases

However, when multivariate analysis was carried out adjusting for time from onset to diagnosis, age at onset, site of onset, sex, attendance at Beaumont clinic, use of riluzole, use of gastrostomy, use of non-invasive ventilation (NIV), the survival effect that was seen on univariate analysis was lost (p=0.189).

Familial cases of ALS are prescribed more riluzole, use more NIV, have gastrostomy tubes inserted more frequently, and attend specialist clinics more frequently than sporadic cases (p=0.011, p<0.0001, p=0.036, p<0.0001).

4.2.15 Results: Survival in ALS by age at disease diagnosis

The 1304 patients were split into six categories depending on their age at diagnosis; 20-39, 40-49, 50-59, 60-69, 70-79, and 80 years and over. Three patients were diagnosed between the age of 15 and 19 years and were not included in this analysis.

Univariate analysis, which was carried out to assess the mean and median survival times from diagnosis to death in each age category, demonstrated that patients diagnosed with the
condition at a younger age had a longer survival time ($p<0.0001$). Multivariate analysis, adjusting for time from onset to diagnosis, site of onset, sex, attendance at Beaumont clinic, family history, use of riluzole, use of gastrostomy, use of non-invasive ventilation (NIV), and time period demonstrated that, for each increasing age group compared to the 20-39 age group, the hazard rate of death increased.

![Cumulative Survival](chart.png)

**Table 4.8** An increased hazard rate of death is associated with older age at diagnosis of ALS

<table>
<thead>
<tr>
<th>Age at diagnosis</th>
<th>Mean survival Months (95% CI)</th>
<th>Median Survival Months (95% CI)</th>
<th>Hazard Rate [of death compared to 20-39 age group]</th>
<th>P value, 95% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-39 years</td>
<td>60.3 (40.4-80.1)</td>
<td>44 (19.1-68.9)</td>
<td>1.0</td>
<td>0.0001, 1.0-2.6</td>
</tr>
<tr>
<td>40-49 years</td>
<td>42.8 (33.1-52.5)</td>
<td>24 (18.1-29.3)</td>
<td>1.6</td>
<td>0.0009, 1.2-2.9</td>
</tr>
<tr>
<td>50-59 years</td>
<td>36.4 (30.5-42.4)</td>
<td>21 (19.0-23.0)</td>
<td>1.9</td>
<td>0.0000, 1.6-4.1</td>
</tr>
<tr>
<td>60-69 years</td>
<td>25.5 (21.5-29.4)</td>
<td>14 (12.4-15.6)</td>
<td>2.6</td>
<td>0.0000, 2.2-5.6</td>
</tr>
<tr>
<td>70-79 years</td>
<td>16.7 (13.9-19.5)</td>
<td>10 (8.0-12.0)</td>
<td>3.5</td>
<td>0.0000, 2.4-6.4</td>
</tr>
<tr>
<td>80+ years</td>
<td>13.6 (10.4-16.8)</td>
<td>8 (5.9-10.1)</td>
<td>3.9</td>
<td>0.0000, 2.4-6.4</td>
</tr>
</tbody>
</table>

**Figure 4.8** An increased hazard rate of death is associated with older age at diagnosis of ALS

**4.2.16 Results: Identifying subgroups within the familial ALS cohort**

84 familial ALS cases were reported to the register in the period 1996-2011. The frequency of age at diagnosis was assessed (see figure 4.8). There was no evidence for a bimodal distribution of onset.
Figure 4.9 Frequency of age at diagnosis in familial cases is normally distributed

The frequency of the time from diagnosis to death in months was assessed. On visual assessment of the histogram there were two distinct subgroups of familial ALS patients; subgroup A and subgroup B (see figure 4.9).

36/86 of the familial ALS patients had undergone cognitive testing: 42% (15/36) were categorized as having normal cognition, 28% (10/36) were categorized as ALS-FTD, 19% (7/36) were categorized as having executive impairment, and 11% (4/36) were categorized as having non-executive cognitive impairment (NECI).

Cognitive patterns were then assessed between the two subgroups, which had been identified in analysis of frequency of the time to death (see figure 4.10).
The distribution of the types of cognitive impairment differs between the two groups, with more ALS-FTD in group A, however due to small numbers this did not reach statistical significance.

Familial ALS cases were also over represented as a proportion of all ALS cases surviving longer than 3 years from disease onset. The rate of familial ALS among all ALS cases was 11.6% in those living longer than three years from onset, compared to a rate of familial ALS of 4.9% in all cases living less than three years form onset (p=0.02). Longer living ALS patients are more likely to have a family history, which may this point toward to a pathological gene which causes a phenotype of ALS with a better survival outcome.

### 4.2.17 Results: Geographic location at time of diagnosis

Each patient's address was converted to a geographic co-ordinate and these were visually plotted on a map of the country. A separate map was created for sporadic cases and familial cases. The maps (figure 4.11) do not correct for underlying population structure, however one would expect to see ten times more sporadic cases in each area where there is one
familial case. The area in map B with the grey circle appears to have an excess of familial cases compared to the same region on map A, which demonstrates sporadic cases. There should be in excess of ten SALS cases for every FALS case.

Figure 4.11 Map with geo-coordinates of 1304 patients diagnosed from 1996-2011 in the Republic of Ireland. Map A, on the left, displays the geo-coordinates for sporadic patients (green dots), and Map B, on the right, familial patients (red dots).

4.2.18 Results: Familial cases per county

In order to quantify the proportion of familial cases from each county we used the Irish ALS Register to investigate the frequency of FALS compared with SALS cases in the 26 counties of the Republic of Ireland. The mean rate of FALS in the island of Ireland for the time period 1996-2011 was 6.4% (+/- standard deviation 5%). Tipperary is the only county to report a rate of FALS greater than two standard deviations above the mean. Almost one-in-five (18%) cases from county Tipperary are reported to be familial (> 2SD=16.4%). Kilkenny is the only other county that has a rate of familial ALS greater than one standard deviation above the
mean. All other counties have rates of FALS that fall within one standard deviation of the mean (see figure 4.12).

<table>
<thead>
<tr>
<th>Proportion of cases that are familial in each county</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tipperary                                           0.18</td>
</tr>
<tr>
<td>Kilkenny                                            0.14</td>
</tr>
<tr>
<td>Cavan                                               0.11</td>
</tr>
<tr>
<td>Longford                                            0.11</td>
</tr>
<tr>
<td>Mayo                                                0.10</td>
</tr>
<tr>
<td>Roscommon                                           0.10</td>
</tr>
<tr>
<td>Louth                                               0.11</td>
</tr>
<tr>
<td>Offaly                                              0.09</td>
</tr>
<tr>
<td>Waterford                                           0.07</td>
</tr>
<tr>
<td>Donegal                                             0.08</td>
</tr>
<tr>
<td>Galway                                              0.06</td>
</tr>
<tr>
<td>Kildare                                             0.08</td>
</tr>
<tr>
<td>Laois                                               0.06</td>
</tr>
<tr>
<td>Meath                                               0.06</td>
</tr>
<tr>
<td>Clare                                                0.05</td>
</tr>
<tr>
<td>Cork                                                0.05</td>
</tr>
<tr>
<td>Dublin                                              0.07</td>
</tr>
<tr>
<td>Sligo                                               0.05</td>
</tr>
<tr>
<td>Kerry                                               0.04</td>
</tr>
<tr>
<td>Limerick                                            0.05</td>
</tr>
<tr>
<td>Carlow                                              0.18</td>
</tr>
<tr>
<td>Leitirn                                             0.00</td>
</tr>
<tr>
<td>Monaghan                                            0.00</td>
</tr>
<tr>
<td>WestMeath                                           0.00</td>
</tr>
<tr>
<td>Wexford                                             0.00</td>
</tr>
<tr>
<td>Wicklow                                             0.00</td>
</tr>
</tbody>
</table>

Figure 4.12 The list on the left demonstrates the rate of familial ALS compared to sporadic ALS per county in Ireland – Tipperary and Kilkenny are over represented; On the right there is a map of familial ALS cases in Ireland with a grey circle superimposed on counties Tipperary and Kilkenny (data from Irish ALS Registry 1996-2011).

4.2.19 Results: Surnames on the register

To identify any surname that may appear more frequently than would be expected by chance among ALS patients on the register, we compared the frequency of the ten most common Irish names with the frequency of male surnames (n=785) on the register to see if any of the names were over-represented (see figure 4.12). For data protection reasons the surnames are numbered one to ten.
<table>
<thead>
<tr>
<th>Surname</th>
<th>Frequency of surname from Irish population census (%)</th>
<th>Frequency of male ALS surnames as a percentage of all males on ALS register [%] (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4</td>
<td>1.5 (0.9-2.7)</td>
</tr>
<tr>
<td>2</td>
<td>1.1</td>
<td>1.6 (0.9-2.8)</td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>1.5 (0.9-2.7)</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>0.9 (0.4-1.9)</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>0.8 (0.3-1.7)</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>1.3 (0.7-2.4)</td>
</tr>
<tr>
<td>7</td>
<td>0.8</td>
<td>0.6 (0.2-1.5)</td>
</tr>
<tr>
<td>8</td>
<td>0.7</td>
<td>1.3 (0.7-1.5)</td>
</tr>
<tr>
<td>9</td>
<td>0.7</td>
<td>0.6 (0.2-1.5)</td>
</tr>
<tr>
<td>10</td>
<td>0.7</td>
<td>0.8 (0.3-1.7)</td>
</tr>
</tbody>
</table>

Table 4.7 The frequency of the top-ten surnames on the Irish ALS Register compared to the frequency of the top-ten Irish surnames.

Surname number three was the only surname on the Irish ALS register which appeared statistically more often on the register than in the general Irish population. The frequency of surname number three on the register was 1.5 (95% CI 0.9-2.7).

(Source A; Matheson, Special Report on Surnames in Ireland, 1981. Source B; Murphy, Studies in Irish Genealogy and Heraldry, 2009. For calculation of frequency of observed versus expected ratio Source B is used.)

4.3 Comparison of ALS epidemiology in Ireland and Northern, 2005-2010

4.3.1 Results: General demographics

511 people from Ireland and 208 people from Northern Ireland were diagnosed with definite, probable or possible ALS by El Escorial criteria in the six-year period from January 1st 2005 to December 31st 2010. Demographic parameters were comparable except for age of onset in females, which was significantly older in Northern Irish females.
8% of Irish ALS patients reported a family history of ALS in the period compared to 6.7% of Northern Irish patients.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Ireland (n=511)</th>
<th>Northern Ireland (n=208)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td>64.7 years +/- 11.4</td>
<td>65.9 years +/- 11.6</td>
<td>0.22</td>
</tr>
<tr>
<td>Male</td>
<td>63.4 years +/- 11.2</td>
<td>62.3 years +/- 11.6</td>
<td>0.54</td>
</tr>
<tr>
<td>Female</td>
<td>66.4 years +/- 11.1</td>
<td>69.3 years +/- 10.5</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Site of Onset</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb</td>
<td>63% (306)</td>
<td>66.5% (135)</td>
<td>0.23</td>
</tr>
<tr>
<td>Bulbar</td>
<td>37% (182)</td>
<td>33.5% (68)</td>
<td></td>
</tr>
<tr>
<td><strong>El Escorial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite</td>
<td>64.6% (317)</td>
<td>20.8% (42)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Probable</td>
<td>21.8% (107)</td>
<td>30.2% (61)</td>
<td></td>
</tr>
<tr>
<td>Possible</td>
<td>12.6% (62)</td>
<td>27.2% (55)</td>
<td></td>
</tr>
<tr>
<td>Suspected</td>
<td>1.0% (5)</td>
<td>21.8% (44)</td>
<td></td>
</tr>
<tr>
<td><strong>Time symptom onset to diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>13.0 months</td>
<td>14.5 months</td>
<td>0.22</td>
</tr>
<tr>
<td>Male</td>
<td>13.4 months</td>
<td>14.0 months</td>
<td>0.74</td>
</tr>
<tr>
<td>Female</td>
<td>12.5 months</td>
<td>14.9 months</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 4.8 Demographic features of ALS patients from Ireland compared to Northern Ireland, 2005-2010

4.3.2 Results: Incidence

The corrected incidence was the same in both regions for the time period; Ireland - 2.6 per 100,000; Northern Ireland - 2.5 per 100,000.
Figure 4.13 Corrected incidence per age category for Ireland compared to Northern Ireland, 2005-2010

Figure 4.14 Corrected incidence for each age category split by sex and country

4.3.3 Results: Prevalence

There was no difference in prevalence between the two time periods.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Ireland 2010</th>
<th>Northern Ireland 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>237 cases</td>
<td>93 cases</td>
</tr>
<tr>
<td>Population &gt; 15 years old</td>
<td>3,311,500</td>
<td>1,444,325</td>
</tr>
<tr>
<td>Population &gt; 15 years old</td>
<td>6.8 per 100,000 (95% CI 5.9-7.6)</td>
<td>6.4 per 100,000 (95% CI 5.1-7.7)</td>
</tr>
</tbody>
</table>
4.3.4 Results: Survival

Age-matched univariate analysis comparing survival time in Ireland and Northern Ireland demonstrated a survival advantage in Irish patients (median survival from diagnosis to death was 14 months in Irish ALS patients compared to 11 months in Northern Irish patients, p=0.0001). Multivariate analysis, adjusting for region (e.g. Ireland, Northern Ireland), time from onset to diagnosis, age at onset, site of onset, sex, family history, use of riluzole, use of gastrostomy, use of non-invasive ventilation (NIV), demonstrated that patients from Ireland had a survival advantage (HR 0.72, 95% CI 0.6-0.87, p=0.001).

If multivariate analysis included attendance at Beaumont clinic, then there was also a demonstrable survival benefit for patients who attended the specialist clinic compared to Irish and Northern Irish patients who did not attend the clinic (HR 0.55, 95% CI 0.46-0.60, p=0.001).

<table>
<thead>
<tr>
<th></th>
<th>Mean (95% CI)</th>
<th>Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ireland (n=508)</td>
<td>23.6 months (21.3-26.0)</td>
<td>14 months (12.5-15.5)</td>
</tr>
<tr>
<td>Northern Ireland (n=206)</td>
<td>16.5 months (14.1-18.8)</td>
<td>11 months (8-14)</td>
</tr>
</tbody>
</table>

Figure 4.15 Cumulative survival for patients from Ireland compared to Northern Ireland (p=0.0001). Legend: Ireland (blue line), Northern Ireland (green line).
4.3.5 Discussion

The analysis demonstrates that a well-maintained and population-representative disease register is invaluable for the reporting of demographic information.

**Incidence:** The incidence of ALS in Ireland is stable over a sixteen-year period at 2.6 per 100,000 for the population over the age of fifteen years. Detailed analysis of the proportion of cases in each age category and the incidence of disease for different age categories over four time periods did not show any significant difference.

**Prevalence:** The prevalence is the same in both time periods, as confidence intervals are overlapping. For a register one must wait at least ten years before one can be confident that the prevalence figures are correct. This is because a small proportion of ALS patients survive longer than 3-5 years. In order to account for these patients, one must wait until the register has been in operation more than ten years.

**Age at disease onset/diagnosis:** Analysis of the ALS register between four time periods over 16 years in total showed a trend toward an increase in age at symptom onset and diagnosis. However this did not translate to an increase in incidence of ALS at extreme age. The reason for this may be that the increase in age at onset is only by two to three years over a sixteen-year period, and incidence intervals are recorded for ten-year intervals.

Because of this trend toward an increasing age of onset in Ireland, and the fact that age of onset varies in different countries I hypothesized that the mean age at onset of ALS was directly proportional to life expectancy in the geographic region that the patient was living, and that this constant proportion may account for all observed variations in age of onset seen in different geographical populations and between historical cohort studies. This hypothesis was tested by calculating the proportion between age at disease onset and life expectancy in the region during the study period, and determining the variation between countries and across time periods. Prospective population based studies were identified and the mean age of onset was extracted from all available publications. In the absence of the age at onset, the mean age at diagnosis was recorded. Life expectancy values for the middle of the study
period were used as a proxy for life expectancy in each region (http://go.worldbank.org). The proportional age at onset was calculated by dividing the mean age at onset (or diagnosis if onset data were unavailable) by the life expectancy for that region at the midpoint of that given study period. 95% confidence intervals were calculated and proportions were plotted to ensure that all values were within the range. Regression analysis was performed to identify linear correlation. Residuals were tested for normality and variance was tested.

Sixteen studies were identified of which eight comprised prospective population based studies that reported either mean age at onset or diagnosis[56-64] and a further eight retrospective population based cohorts were identified, and the proportional age of onset was calculated for each study[42, 65, 67-69, 71, 73, 75, 208-210].

Figure 4.16 Figure A shows proportional age at onset of ALS, and figure B shows proportional age at diagnosis of ALS

A scatterplot was generated representing mean age at onset on the x-axis and life expectancy in the region during the study period on the y-axis. A linear pattern was noted and regression analysis was performed to test for correlation.
Regression analysis revealed that the mean age at onset correlated positively with local life expectancy at the midpoint of the study \( (r=0.91, p=0.01) \). This implies that life expectancy in the region at the time of onset explained 83% of the variance in age of onset of ALS \( (r^2=0.83) \).

In developed countries, overall life expectancy is primarily dependent on a combination of genetic determinants that increase risk for certain life-shortening conditions (e.g. hypertension, lipid profile); and modifiable factors including diet, smoking, quality of life, and medical service provision. The finding that there is a constant relationship between the mean age at onset of ALS and life expectancy may suggest one of two things; firstly one could argue that an increase in life expectancy would lead to more people at extreme age developing ALS, with an increase in mean age, and secondly it may be argued that the factors that influence life expectancy of a population also influence the onset of ALS. The argument that an ageing population leads to an increased in age at onset is unlikely as the peak incidence of ALS falls
after the age of 70. The second argument, that the factors that influence better ageing also prevent ALS at a younger age may be explained by increases in biological reserve which cause people to develop ALS at an older chronological age.

Our data suggest that Scotland is an outlier with a higher proportional age at disease onset. However, Scotland also has a lower life expectancy than its neighbours on the British Isles, primarily due to the high cardiovascular mortality rate. Recently it has been suggested that there is an inverse relationship between cardiovascular risk and the risk of developing ALS, which could account for the relatively higher proportion of 0.9 between age of onset of ALS and life expectancy in Scotland.

This study has limitations. The life expectancy used in calculating the proportional age at onset is a proxy for life expectancy at birth for each patient, and there are insufficient published data to permit further analysis of cohorts gender. However, the observation that the relationship between age of disease onset and life expectancy is constant across populations is compelling, and argues in favour of complex genetic risk linked to the process of biological ageing.

Survival: Survival analysis was carried out looking at the time from diagnosis to death or censoring. This time interval was chosen because patient entry onto the register is at diagnosis and not onset; therefore if 'onset to death' were used this would introduce positive survival bias into the earliest cohort, with preferential recruitment of patients who had a longer time between onset and diagnosis e.g if a patient had onset in July 1995 and was diagnosed in October 1995, then they would not be included in this 16-year cohort, compared to patients with symptom onset in July 1995 and diagnosis in July 1996, who has a better prognosis. Therefore before survival analysis was carried out on this cohort, an apriori decision was made to analyze survival length from time of diagnosis to time of death or censor, and to co-vary for the time from onset to diagnoses in all analyses.

Survival analysis was carried out in a second way to look specifically for a period effect, but no period effect could be identified.
There was no demonstrable survival benefit between the four time periods, despite the introduction of a number of interventions. NIV was associated with a poor outcome; this is due to the association of need for NIV with decreased respiratory function and therefore poor prognosis.

A survival benefit among patients prescribed riluzole was demonstrated, and also for those patients attending a specialist ALS clinic. Multivariate analysis was used to co-vary for factors that may differentiate a patient who attends a specialist clinic from those who do not, including time from symptom onset to diagnosis. The increase in survival is likely to be due to the attention of specialist ALS practitioners who introduce and manage interventions such as gastrostomy and non-invasive ventilation at the correct time, physiotherapists who advise on respiratory exercises to maintain ventilation and manage secretions, speech and language therapists who advise on swallow safety, dieticians who work with the patient and family to maintain adequate weight, an occupational therapist who ensures safety at home, and ALS nurse specialists who can advise the patient and their family on relevant issues and carry out home visits.

One possible reason for the failure to see an increase in survival may be that the available biological reserve declines in patients with an older age of disease onset, and that the age of onset increases over the time period of the study and this increase is not adequately controlled for using the Cox proportional hazard method. This hypothesis would predict that people who develop ALS at extreme old age would have a shorter disease course. Data from the Irish Register supports this and the hazard rate for death is four times higher in the oldest age category compared with the youngest age category. Adjustment for the age of disease onset is routinely applied in survival models, but ‘biological reserve’ is not routinely taken into account. An increase in the mean age in onset of ALS could therefore mask a small survival benefit of less than a year when comparing historical cohorts with different life expectancies. This may in part explain the absence of an observed improvement in survival.
between period cohorts despite the use of interventions such as non-invasive ventilation and the introduction of riluzole.

**Comparison of Ireland to Northern Ireland:** A political divide separates Ireland and Northern Ireland; which means that the hospital systems and ALS registries are entirely independent of each other. Despite this the rates of capture and epidemiological features are remarkably similar. However survival time in Northern Ireland is shorter than in the Republic of Ireland. There is no obvious reason for this discrepancy, which exists even after co-varying for all factors, although one could speculate that it may be because Northern Ireland was without a specialist ALS clinic until 2010. Another possible reason for the differences reported may relate to differences in underlying population substructure which may have been influenced by the settlement pattern of Northern Ireland.

**Familial ALS:** The reported rate of familial ALS was ~6%, increasing to ~8% in the latter two time periods. This is likely to be due to the vigilance for recording family history properly on the register.

Patients with a family history of ALS have an earlier age at symptom onset and are diagnosed at a younger age; this is likely to be due in the most part to a genetic effect which drives an earlier age of onset, but may also be due to a clinician diagnosing ALS more quickly in a person with signs and symptoms and a strong family history, than in a person with no family history. Patients with familial ALS also receive more interventions than patients with sporadic disease. This finding may be because they are more likely to be aware of the disease and may have experience of interventions used in relatives, and are therefore more likely to ask their clinician about the intervention and also may be more likely to attend a specialist centre with greater access to specialist intervention. Multivariate analysis, correcting for multiple interventions and an earlier age of onset, failed to identify any difference in survival between sporadic and familial cases.

Two methods were used to demonstrate a higher than expected rate of familial cases in the Tipperary/Kilkenny region. The first method involved plotting geographic location of sporadic and familial cases on a map of Ireland. This map did not correct for underlying
population structure, however it is visually demonstrated that there are more familial cases than would be expected in this region of Tipperary/Kilkenny, as one would expect to see ten sporadic cases for every familial case. This was confirmed by estimating the proportional rate of familial ALS in each county; the rate in Tipperary was 18%, which is more than two standard deviations above the national rate. Genetic samples from familial patients in these regions may prove fruitful in future genetic studies.

Surname number three was also demonstrated to be over represented in male patients on the register, perhaps because of a small genetic founder effect.

**Identification of subgroups within familial ALS cases:** Epidemiological methods were applied to the ALS register with the aim of identifying subgroups that may exist within the familial ALS cohort. Preliminary analysis was undertaken in early 2011, nine months prior to the discovery of the \( C9orf72 \) repeat expansion. It was evident from looking at survival data that a bimodal distribution in survival time existed. When results of cognitive testing were split to reflect the two groups identified, it became apparent that the former group with shorter survival had more evidence of cognitive impairment, in particular ALS-FTD. The next chapter describes how data, from the ALS register and information from a longitudinal study on cognition in ALS, was used to characterise the phenotype of patients with the \( C9orf72 \) repeat expansion. It will become evident that the subgroup of familial ALS patients with shorter survival and a high burden of cognitive impairment identified through this epidemiological study, likely represents the subgroup of familial ALS patients with the \( C9orf72 \) repeat expansion.

This finding highlights the usefulness of an ALS register in the process of sub-phenotype recognition, and subsequently gene discovery.
Chapter 5 A population-based cohort study to describe the phenotype of patients with ALS carrying a C9orf72 repeat expansion

5.1 Introduction

The repeat C9orf72 expansion, associated with ALS and FTD, was first described in September 2011[147, 148]. Data from the Irish ALS register and longitudinal study of cognition was used to characterise the phenotype of ALS associated with the C9orf72 repeat expansion.

5.2 Aims

Specific aims:

1. To use data from the Irish ALS Register to describe the phenotype of ALS in patients carrying the C9orf72 repeat expansion
2. To examine the cognitive phenotype and radiological features associated with the C9orf72 repeat expansion in ALS patients
3. To create an algorithm which is clinically useful, to determine which ALS patients warrant testing for the repeat C9orf72 repeat expansion

5.3 Methods

5.3.1 Sample Selection

435 high quality patient samples were selected for screening from the DNA bank. Of the 435 patients, 191 patients are population-based incident cases partaking in a longitudinal study of cognition in ALS. 188 age-, sex-, and, geographically-matched controls were also selected.

5.3.2 Genetic testing hexanucleotide repeat expansion in C9orf72

435 DNA samples were screened using repeat-primed PCR for the presence of a GGGGCC repeat expansion. Primer sequences used for the PCR stage were:

Forward: 6-FAM/AGTCGCTAGAGCGGAAAGC

149
The PCR assay was performed in a reaction volume of 38ul, containing 9ul DNA (5nm), 14ul Faststart PCR Master Mix (Roche), 5ul 7-Deaza-dGTP (Roche), 1 x Q Solution (Qiagen), 7% DMSO (Sigma-Aldrich), 0.9mM MgCl2 (Qiagen), 0.7 uM reverse primer consisting of 4 ‘GGGGCC’ repeats with an anchor tail, 1.4uM 6FAM-fluorescent labelled forward primer. A touchdown PCR cycling programme was used, with the annealing temperature gradually lowered from 70°C to 56°C in 2°C increments, and a 3 minute extension time for each cycle. PCR products were analyzed on an Applied Biosystems 3130xl genetic analyzer and visualized using GeneMapper software (version 4.0). Patients with the characteristic appearance of the expanded hexanucleotide repeat on repeat-primed PCR consisting of a decaying series of 24 or more peaks were regarded as having a positive repeat expansion, as described in the paper by DeJesus-Hernandez and colleagues[147]. All results were found to be reproducible.

5.3.3 Mutation Screening for known genes

For screening of known mutations target-enriched sequencing libraries were prepared from genomic DNA using standard protocols (Agilent SureSelect) and sequenced on an Illumina Genome Analyzer II. Sequence data were aligned and processed using BWA, SAMtools, Picard and GATK to generate variant calls that were filtered to exclude dbSNP132 polymorphisms.

5.3.4 Population-based cohort of incident ALS cases (n=191)

191 population-based incident patients diagnosed from November 2006 to May 2011, selected through the register, have been enrolled in a prospective longitudinal study of cognitive and behavioural function (see figure 5.1 for enrollment flow chart). Detailed longitudinal clinical, neurocognitive, and behavioural data, structural magnetic resonance (MR) imaging, and survival data have been collected on this cohort, and DNA has been banked for genomic analysis. Details of patient ascertainment to the Irish register, and
Criteria for inclusion to the longitudinal study of cognition have been described in the introduction (section 4.2.1).

![Flow chart showing the composition of the cohort of 191 cases recruited from the Irish ALS Register.](chart)

Where possible, patient had neurocognitive testing carried out as part of a separate study, by Dr Marwa Elamin. Cognitive assessment included the following tests:

**Brixton Spatial Anticipation task:** This is a non-verbal test that measures the ability to detect and follow rules and rule changes using a sequence of visuo-spatial stimuli.

**Verbal Fluency:** This is a measure of the number of things a patient can name beginning with a particular letter in a set time period.

**Stroop Colour Word Interference Task:** This is an executive test in which the subjects were presented with a multi-coloured list of names of colours, and asked to simply read the printed words as quickly as they can.
**Backward Digit Span:** The digit span is a commonly used test of attention, working memory, and mental processing capacity, where patients are asked to read out a sequence of numbers backwards.

**Logical Memory:** Logical Memory examines the ability to recall and recognise multiple elements in a logical story sequence.

**Wechsler Test of Adult Reading:** This study estimates the subjects' pre-morbid intelligence quotient. The test that comprises 50 words with irregular pronunciations. As standard pronunciation rules do not apply, to read the words correctly the subject must have prior knowledge of the words.

**Frontal Systems Behaviour Scale (FrSBe):** Information on behaviour was obtained via direct assessment of the patient, and semi-structured interviews with the carers using the FrSBe questionnaire. The FrsBe scale is a Self-Rated form (to be filled by the subject) and a Family Rated Form (to be completed by subject's carer).

### 5.3.5 Imaging

Grey matter voxel based morphometry (VBM) analysis was performed in ten patients with and 30 without the repeat expansion. The demographic details (age, disease duration and clinical phenotype) of the two groups were matched. A study specific template was created, to which the grey matter images from each subject were non-linearly co-registered. A voxel wise generalized linear model (GLM) was used to test for differences between the group with the repeat expansion and the group without the repeat using permutation-based non-parametric testing. A minimum cluster size of 800 microliters was applied to the results of the analysis to highlight only significant regions of differences. Statistical significance was set at $p < 0.01$ (voxel level) and corrected for multiple comparisons at $p < 0.05$ (family wise error).
5.3.6 Statistical methods

Demographic and clinical characteristics of the participants are reported as percentages for categorical variables and means/medians for continuous variables. Comparisons were made using Chi-square or Fisher Exact test or 2-sample t-test as appropriate. For group analysis where the samples were small the p-value was calculated using the Montecarlo method. Where variables were normally distributed a mean is reported. Where variables were not normally distributed a median is reported and non-parametric tests were used. Univariate analysis of covariance (ANCOVA) was carried out to adjust for confounding factors where appropriate. Spearman's Rho test was used when assessing the degree of correlation strength between variables with a non-parametric distribution. Survival time was defined as time from diagnosis to death, covaried for time from onset. Time from symptom onset to diagnosis was compared between groups to ensure lack of lead-time bias. Patients who were alive at the time of analysis were censored. Univariate assessment of the survival effect of categorical variables was carried out using Kaplan-Meier (KM) survival analysis and equality of outcome was assessed using the log-rank test. Cox proportional hazards method was used for multivariate survival analysis using backward elimination (Wald test), estimation of hazard ratio (HR), and 95% confidence intervals. Logistic binary regression was used to ascertain predictive factors associated with patients testing positive for the repeat expansion. All tests were 2-tailed and statistical significance was set at p<0.05. Statistical analysis was carried out using SPSS version 18 (SPSS Inc, Chicago, IL).

5.4 Results

5.4.1 Genetic testing hexanucleotide repeat expansion in C9orf72 (n=435)

Of 435 banked DNA samples derived from the Irish ALS Register, 9% (39/435) had the characteristic appearance of a GGGGCC hexanucleotide expansion consisting of a decaying series of 24 or more peaks. All positive samples had 30 or more repeats. Figure 5.2 demonstrates a positive pattern.
The frequency of the repeat expansion was 41% (20/49) in patients with familial ALS (FALS), and 5% (19/386) in apparently sporadic cases. No expansions above 23 repeats were noted in 188 healthy controls (see figure 5.3).

In analysis of the raw data the mean number of repeats for the 396 patients who were repeat negative (fewer than 30 repeats) was 5.6 with a standard deviation of 3.6 (95% CI 0-12.8). We compared the ages of onset and diagnosis in those negative for a repeat expansion with a
repeat number higher than two standard deviations above the mean (12.8), compared to those who fell within the 95% standard deviation range (<12.8). Therefore the cut off was set at 2-12 and 13-22.

Figure 5.4 Repeat number in 396 patients who did not carry an expanded repeat

A. Frequency and distribution of repeats in 396 patients deemed negative for pathological expansion (23 or fewer repeats)

B. Scatter plot demonstrating the relationship between age at diagnosis and repeat number. A negative correlation can be seen between age at diagnosis and repeat number in patients with 13-23 repeats.

C. Spearman (Rho) correlation coefficients demonstrate correlation between age at disease onset and diagnosis in patients with 13-23 repeats.
5.4.2 Population-based cohort of incident ALS cases (n=191)

Detailed phenotypic data were available on 44% (191/435) of samples from the DNA bank. This cohort of patients represented a population based incident group who had participated in a longitudinal case control study of cognition and behaviour in ALS, which has been running since 2006. Analysis of sequence data generated for SOD1, TARDBP and FUS revealed no known or potentially novel pathogenic variants in these patients. 11% (21) of the 191 patients in this cohort carried the C9orf72 repeat expansion.

The age of disease onset was younger in patients with the repeat expansion, and there was no difference between those with the repeat expansion and those without with respect to the time from symptom onset to diagnosis (14.7 versus 13.2 months, p=0.541). There was no statistically significant difference in gender, site of onset, or ALS Functional Rating Scale (ALSFRS) at first assessment between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Patients without repeat expansion=170</th>
<th>Patients with repeat expansion n=21</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at onset of disease</td>
<td>61.3 years (SD 10.6 years)</td>
<td>56.3 years (SD 8.3 years)</td>
<td>p=0.043</td>
</tr>
<tr>
<td>Median age at onset of disease</td>
<td>61.9 years (IQR 56.4-67.9 years)</td>
<td>57.5 years (IQR 50.5-63.5 years)</td>
<td>p=0.019</td>
</tr>
<tr>
<td>Mean age at diagnosis of disease</td>
<td>62.5 years (SD 10.6 years)</td>
<td>57.5 years (SD 8.1 years)</td>
<td>p=0.041</td>
</tr>
<tr>
<td>Median age at diagnosis of disease</td>
<td>62.8 years (IQR 57.1-69.1 years)</td>
<td>59.6 years (IQR 51.9-64.2 years)</td>
<td>p=0.015</td>
</tr>
<tr>
<td>Site of onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar</td>
<td>33.5% (57)</td>
<td>33.3% (7)</td>
<td>p=0.822</td>
</tr>
<tr>
<td>Spinal</td>
<td>66.5% (113)</td>
<td>66.7% (14)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>59.4% (101)</td>
<td>47.6% (10)</td>
<td>p=0.353</td>
</tr>
<tr>
<td>Female</td>
<td>40.6% (69)</td>
<td>52.4% (11)</td>
<td></td>
</tr>
<tr>
<td>Mean ALSFRS at first assessment</td>
<td>36.4 (SD 8.1)</td>
<td>33.8 (SD 6.7)</td>
<td>p=0.181</td>
</tr>
</tbody>
</table>

Table 5.1 Demographic information for ALS patients from the population based cohort (n=191) with the C9orf72 repeat expansion compared to patients without the repeat expansion

5.4.3 Family history in patients with the C9orf72 repeat expansion

Of the 435 samples tested, 49 had reported a family history of ALS. Almost half, 41% (20/49) of the FALS patients carried the repeat expansion.
From the population-based cohort of 191 patients, 24 reported a family history of ALS. 12/24 patients from this cohort (191) with a family history of ALS carried the repeat expansion. Six of the patients with a repeat expansion had a verified first-degree or second-degree relative with FTD. Of those who did not carry the C9orf72 repeat expansion (170 patients) only three (2%) described a family history of FTD (p<0.0001).

Of the three remaining patients carrying the repeat expansion who were apparently "sporadic", one had a strong family history of depressive illness with suicide; one had a strong family history of parkinsonism in two paternal siblings, as well as unspecified dementia and mobility problems in a paternal grandmother, and one was unable to provide a family history as both parents had died prematurely.

5.4.4 Disease progression

Disease progression in patients who had undergone second neuropsychological assessment (n=91) was calculated using the ALS Functional Rating Scale (ALSFRS) by dividing the decline in functional scores over the time between the two assessments. Compared to patients without the repeat expansion, (n=82), those patients with the repeat expansion (n=9) had a significantly faster rate of motor progression (a median decline of 0.83 [IQR 0.34-1.43] versus 1.54 [IQR 107-2.08] points in total ALSFRS-R scores/month, p=0.009). This difference was driven by a higher rate of decline in spinal function (1.2 [IQR 0.77-1.53] versus 0.5 [IQR 0.17-0.83] points decline in ALSFRS spinal subscore/month, p=0.016).

The presence of the C9orf72 repeat expansion generated a significant hazard rate (HR 1.9, 95% CI 1.1-3.7, p=0.035) on multivariate analysis after adjusting for age of symptom onset, gender, time from onset to diagnosis, and site of onset. Multivariate analysis of the ALS patients with behavioural variant frontotemporal dementia (bvFTD) revealed that the presence of the repeat expansion was associated with a hazard ratio of 3.7 (95% CI 1.1-12.3, p=0.034).
Figure 5.5. Kaplan survival probabilities for patients with Amyotrophic Lateral Sclerosis (ALS) stratified for the presence of the repeat expansion.

A. Kaplan-Meier survival probabilities for all ALS patients in the population-based cohort (n=191) [Multivariate regression gives a hazard ratio of 1.9 for ALS patients with the repeat expansion; 95%CI 1.1-3.7, p=0.035]

B. Kaplan-Meier survival probabilities for ALS patients in the population-based cohort with bvFTD (n=25) [Multivariate regression gives a hazard ratio of 3.7 for bvFTD-ALS patients with the repeat expansion; 95%CI 1.1-12.3, p=0.034]

(Blue solid line: Patients who did not carry the repeat expansion; Green dotted line: Patients who carried the repeat expansion)

A multivariate comparison of survival from diagnosis to death was also made between FALS cases carrying the C9orf72 repeat expansion and FALS cases negative for the C9orf72 repeat expansion. Familial ALS cases without the repeat expansion lived significantly longer than familial cases with the repeat expansion (median 28 versus 13 months, p=0.015).
### Table 5.6

<table>
<thead>
<tr>
<th></th>
<th>Mean (95% CI)</th>
<th>Median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9orf72 negative (n=42)</td>
<td>59 months (36-82)</td>
<td>28 months (13-43)</td>
</tr>
<tr>
<td>C9orf72 positive (n=25)</td>
<td>18 months (13-24)</td>
<td>13 months (11-15)</td>
</tr>
</tbody>
</table>

**Figure 5.6** Kaplan-Meyer survival curves demonstrating significantly longer survival in the familial cases of ALS without known genes compared to familial cases carrying the C9orf72 repeat expansion (p=0.015)

(Blue line: Patients who did not carry the repeat expansion; Green line: Patients who carried the repeat expansion)

#### 5.4.5 Cognitive profile

All patients in the population-based incident cohort (n=191) had undergone detailed neuropsychological testing. 186 were classified into one of four cognitive categories (ALS-FTD, executive impairment, non-executive impairment and normal cognition). Five of the 191 patients were too unwell to complete the full cognitive battery and were therefore not classified according to cognitive criteria. However, behavioural data were available from all five, and these data were included in the analysis.

Of the 186 patients who completed full cognitive testing at the time of first assessment, 91 underwent further testing after a six-month interval and 34 had a third assessment after an interval of one year from initial assessment.
Detailed behavioural data were available using the Frontal Systems Behavioural Scale (FrSBe) on 68.1% (130/191) of the cohort.

Patients with the repeat expansion exhibited a characteristic cognitive profile, with a significantly higher frequency of co-morbid frontotemporal dementia.

Figure 5.7 Neuropsychological test results from ALS population-based cohort (n=186)

Statistical comparison between groups of the outcome of neuropsychological testing on ALS patients without the repeat expansion compared to patients with the repeat expansion (p=0.0002). [Key: ALS-FTD: Amyotrophic lateral sclerosis with co-morbid frontotemporal dementia, NECI: non-executive cognitive impairment]

Univariate ANCOVA analysis compared performance on executive tasks (Category fluency, Verbal fluency Index, Brixton Spatial Anticipation task, Stroop Interference task, and Backward digit span tests) between patients with the repeat expansion and those without, after adjusting for differences in age at time of assessment. Patients with the repeat expansion had significantly more executive impairment (Brixton test, p=0.043).
Table 5.2 Difference in tests of executive function between repeat-positive and repeat-negative ALS patients

[* lower score indicates worse outcome, ** higher score is worse outcome]

<table>
<thead>
<tr>
<th>Test</th>
<th>Patients without repeat expansion</th>
<th>Patients with repeat expansion</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category fluency* (mean)</td>
<td>16.5 (SD 7.9) [n=126]</td>
<td>15.1 (SD 7.9) [n=14]</td>
<td>p=0.1</td>
</tr>
<tr>
<td>Combined verbal fluency index** (median)</td>
<td>13.8 (IQR 7.7-27.0) [n=149]</td>
<td>17.8 (IQR 11.3-48.7) [n=19]</td>
<td>p=0.056</td>
</tr>
<tr>
<td>Brixton scaled score* (mean)</td>
<td>5.1 (SD 2.3) [n=154]</td>
<td>4.6 (SD 2.8) [n=19]</td>
<td>p=0.043</td>
</tr>
<tr>
<td>Stroop i) CW score* (mean)</td>
<td>69.3 (SD 33.3) [n=119]</td>
<td>60.3 (SD 32.5) [n=14]</td>
<td>p=0.067</td>
</tr>
<tr>
<td>Stroop ii) SEF score** (mean)</td>
<td>37.1 (SD 25.9) [n=117]</td>
<td>44.5 (SD 25.6) [n=12]</td>
<td>p=0.054</td>
</tr>
<tr>
<td>Backward digit span* (mean)</td>
<td>10.6 (SD 3.5) [n=117]</td>
<td>9.7 (SD 3.7) [n=12]</td>
<td>p=0.221</td>
</tr>
</tbody>
</table>

5.4.6 Cognitive profile of Patients with co-morbid frontotemporal dementia (ALS-FTD)

Further subgroup analysis was carried out in the ALS-FTD group (n=30) to identify differences in the phenotype of dementia. ALS-FTD patients with the repeat expansion (n=10) had symptoms of ALS at a younger age, were more likely to have spinal onset, and to have behavioural variant FTD (bvFTD) (see table 5.3). A higher mean language score on the Boston Naming Test (20.1 versus 13.5, p=0.043) was noted.
### Table 5.3 Demographic information for ALS-FTD patients with the repeat expansion compared to ALS-FTD patients without the repeat expansion from the population-based cohort (n=30)

<table>
<thead>
<tr>
<th></th>
<th>Repeat-negative patients with ALS-FTD (n=20)</th>
<th>Repeat-positive patients with ALS-FTD (n=10)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at onset of disease</td>
<td>64.8 years (SD 9.5 years)</td>
<td>57.4 years (SD 8.1 years)</td>
<td>p=0.045</td>
</tr>
<tr>
<td>Median age at onset of disease</td>
<td>65.6 years (IQR 56.7-72.5 years)</td>
<td>58.2 years (IQR 55.9-62.8 years)</td>
<td>p=0.100</td>
</tr>
<tr>
<td>Mean age at diagnosis of disease</td>
<td>65.9 years (SD 9.7 years)</td>
<td>58.7 years (SD 7.9 years)</td>
<td>p=0.053</td>
</tr>
<tr>
<td>Median age at diagnosis of disease</td>
<td>66.9 years (IQR 57.5-74.2 years)</td>
<td>59.8 years (IQR 56.9-63.9 years)</td>
<td>p=0.100</td>
</tr>
<tr>
<td>Site of onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar</td>
<td>50% (10)</td>
<td>10% (1)</td>
<td>p=0.049</td>
</tr>
<tr>
<td>Spinal</td>
<td>50% (10)</td>
<td>90% (9)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65% (13)</td>
<td>70% (7)</td>
<td>p=1.000</td>
</tr>
<tr>
<td>Female</td>
<td>35% (7)</td>
<td>30% (3)</td>
<td></td>
</tr>
<tr>
<td>Type of FTD</td>
<td>75% bvFTD (15)</td>
<td>100% bvFTD (10)</td>
<td>p=0.143</td>
</tr>
<tr>
<td></td>
<td>25% language variant (5)</td>
<td>0% language variant (0)</td>
<td></td>
</tr>
</tbody>
</table>

**5.4.7 Behavioural profile**

Patients with the repeat expansion who had normal cognition exhibited a higher rate of behavioural impairment compared to patients without the repeat expansion (66.6% (4) versus 22% (11), p=0.038). Repeat-positive patients also had a significant increase in apathy scores (p=0.032), and dysexecutive behaviour (p=0.023), (see table 5.4).
Table 5.4 Differences in behavioural scores changes on FRSBE test between repeat-positive and repeat-negative ALS patients. Higher scores indicate more behavioural impairment.

Of the 21 patients carrying the expanded hexanucleotide repeat only two (9.5%) were classified as having neither cognitive impairment or behavioural impairment at the time of their first assessment. Follow up testing was not available for either patient, and both had strong family histories of ALS and FTD respectively (three or more first- or second-degree relatives affected in each pedigree).

None of the 63 patients who were behaviourally normal, and had a negative family history, carried the expanded hexanucleotide repeat.

5.4.8 Imaging

Grey matter voxel based morphometry (VBM) analysis was performed on a cohort of ten ALS patients with the repeat expansion and 30 age and disease duration matched ALS patients without the repeat expansion (see table 5.5).
<table>
<thead>
<tr>
<th></th>
<th>Patients without repeat expansion (n=30)</th>
<th>Patients with repeat expansion (n=10)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at onset of disease</td>
<td>59.4 years (SD 10.0 years)</td>
<td>54.0 years (SD 9.8 years)</td>
<td>p = 0.151</td>
</tr>
<tr>
<td>Mean disease duration at time of scan</td>
<td>26.0 months (SD 22.1 months)</td>
<td>26.1 months (SD 10.5 months)</td>
<td>p = 0.985</td>
</tr>
<tr>
<td>Male patients (number)</td>
<td>14/30</td>
<td>8/10</td>
<td>p = 0.082</td>
</tr>
<tr>
<td>Right handed (number)</td>
<td>27/30</td>
<td>7/10</td>
<td>p = 0.153</td>
</tr>
</tbody>
</table>

*Table 5.5 Demographic information for ALS patients with the repeat expansion compared to ALS patient without the repeat expansion who underwent 3-T MR imaging*

Statistically significantly increased grey matter atrophy was present in the cohort carrying the repeat expansion in the right inferior frontal gyrus, right superior frontal gyrus, left anterior cingulate gyrus, and the right precentral gyrus (see figure 5.8).
5.4.9 Assessment of risk

Predictive modeling was carried out using direct logistic regression to assess which factors were predictive for harbouring the repeat expansion. The final model contained the following predictors: i) behaviour at first assessment, ii) family history of ALS, and iii) family history of FTD. 191 cases were included in the analysis but 79 did not have complete behavioural data available from first assessment and were not included (n=112 included in analysis). The strongest predictor for the presence of a C9orf72 repeat expansion among ALS patients was a family history of FTD, yielding an odds ratio of 102.2 (95% CI 12.3-845.5, p<0.0001). A family history of ALS (defined as at least one first or second degree relative with ALS) was also a strong predictive factor with an odds ratio of 30.8 (95% CI 6.8-138.5, p<0.0001). Abnormal behaviour (as defined by abnormal FrSBe score) at first assessment generated an odds ratio of 4.9 (95% CI 1.2-19.9, p=0.027).
A clinical screening tool was then generated using the predictive factors that had been generated. The algorithm was then applied to the clinical cohort of patients, for whom data was available, to calculate sensitivity and specificity of the screening method.

If genetic testing was offered to all patients with a family history of ALS or FTD, and also to those patients without a family history but with behavioural impairment, and not offered to those with no family history of ALS or FTD and no behavioural impairment, the sensitivity of the screening algorithm was 100% (95% CI 0.85-1.0) and the specificity was 67% (95% CI 0.57-0.76). The high sensitivity is due to the fact that no patient with normal cognition and behaviour, who had a negative family history, had the repeat expansion (63 patients). Validation of this algorithm in a larger cohort will be necessary.

**Figure 5.9 Screening algorithm (figures shown are actual numbers from our population based incident cohort)**

### 5.5 Discussion

This is the first detailed population-based study of the C9orf72 hexanucleotide repeat expansion in ALS patients. Using a representative sample of 435 cases from the Irish ALS...
DNA bank, 41% (20) of Irish patients with a family history of ALS and 5% (19) of the apparently sporadic population had the repeat expansion.

Careful analysis of the output of the reverse prime PCR process using GeneMapper software (version 4.0) is very important in determining whether the sample is positive or negative. The mechanism by which reverse prime PCR replicates the sample means that a sample with many hexanucleotide repeats will result in the characteristic pattern of a decaying series of peaks that run on beyond 24 peaks. In the author's experience samples that are positive will run on well beyond 35 in positive samples (using the stated protocol). In summary, a positive result will have more than 24 repeats and also an exponential decline in the number of peaks seen (figure 5.2). If a sample is heterozygous for differing lengths of repeat (either of normal or pathological length), it may be possible to count the number of repeats in each allele. Any indeterminate sample results should be repeated for verification.

Samples with more than 24 repeats are associated with a particular phenotype in most cases. In order to explore the relationship between repeat number and pathogenicity further we looked to see if there was any correlation between repeat number within the 'normal range' and demographic factors. We discovered that there was a moderate negative correlation between age of disease onset and diagnosis with repeat number in those with 13-23 repeats indicating a dose effect rather than a threshold effect. As the number of samples in the group with 13-23 repeats was small compared to the 2-12 repeat group, this analysis is not statistically sound as there are many cases in the range 2-12 and very few cases in the range 13-23 repeats. A larger data set with more statistical power is required to look at the relationship between repeat number in the normal range (currently set at 2-23, but may change) and clinical phenotype.

More research is required to determine the actual cutoff for pathogenicity of the C9orf72 repeat expansion. Both ALS patients from the DNA bank who have 22 repeats and who are not currently classified as carrying the expanded hexanucleotide repeat, are cognitively and behaviourally impaired, each had an age of disease onset which was younger than the mean age observed in the repeat expansion carrier group (56.3 years) and both have a family
history of FTD. Going forward we need to look carefully at the patients with 13-23 repeats to investigate the possibility that a repeat length within this range interacts with other genetic or environmental factors to increase susceptibility to ALS or FTD.

Many recent discussions of ALS and FTD have suggested that these conditions form two ends of a disease spectrum. However, detailed cognitive assessment of our 191 population based incident cases[7, 8] suggests that ALS patients are clinically heterogeneous, and can be divided into at least two broad categories: i) ALS patients with 'pure sporadic ALS' (no cognitive or behavioural impairment and no reported family history of ALS or FTD), and ii) ALS patients with a predominance of executive cognitive impairment and behavioural change, with a high proportion of the latter group carrying the repeat expansion. Patients with an expanded repeat had a younger age of onset, more rapid disease progression and shorter survival. This observation has important implications for patient stratification in clinical trials, which at present segregate patients for site of onset, severity of disease and clinical status but not on a prognosis determined by genetic status. As described previously certain genetic mutations are associated with specific phenotypes of ALS. Patients carrying these mutations may have a particularly long survival (SOD D90A) or very poor prognosis (FUS), depending on the specific mutation.

Patient stratification based on genetic status also has potential implications for drug efficacy. Patients with the repeat expansion had significant extra-motor cortex changes on high resolution 3 Tesla structural neuroimaging, including reduced grey matter volume in the right inferior and superior frontal regions, and the left anterior cingulate gyrus. These imaging changes correlated with the more extensive neuropsychological findings in patients with the repeat expansion, which included increased apathy, increased dysexecutive behaviour, and worsened executive function, particularly on the Stroop Interference task, phonemic verbal fluency, and Brixton spatial anticipation task. Apathy has been consistently linked to the anterior cingulate gyri[211] as have the Stroop interference task and the Verbal fluency task[212]. Behavioural change was also a prominent feature in patients with the repeat expansion. More extensive imaging and autopsy studies will be required to further
characterise in detail the structural and neuropathologic differences between patients with and without the repeat expansion.

The prominence of cognitive and behavioural impairment in patients with the repeat expansion has implications in the development of clinical care pathways and also for education and support of carers, as cognitive and behavioural changes affect patients' ability to cooperate with symptomatic treatments including NIV, and increased carer burden.

A positive family history of FTD or ALS had the highest predictive values for the presence of a C9orf72 repeat expansion. This study has demonstrated the importance of accurate and detailed information about family history. In our cohort of 191 patients, 8 (4.7%) patients originally categorized as "sporadic" were subsequently found to have familial ALS based on extensive pedigree interrogation. In fact in the remaining three apparently 'sporadic cases'; one had a strong family history of depressive illness with suicide, and another had a strong family history of parkinsonism in two paternal siblings, as well as unspecified dementia and mobility problems in a paternal grandmother. This suggests that psychiatric and other neurodegenerative diseases may be associated with the repeat expansion.

It is important to take a thorough family history from a reliable source in all patients diagnosed with ALS. If a patient has cognitive impairment the history must be taken from another relative. Identification of FTD in preceding generations is challenging, as clinical recognition of the condition is relatively recent. In this cohort of patients, 9 of 191 (4.7%) had no family history of ALS, but had a strong family history of FTD: six of these patients carried a repeat expansion, and of the three who did not carry a repeat expansion (>24 repeats), two had 22 repeats.

Survival in familial ALS patients with the C9orf72 repeat expansion is reduced, compared to familial ALS patients without a repeat expansion. In 4.2.16, in analysis carried out prior to the discovery of the repeat expansion, I demonstrated the presence of two distinct subgroups within the cohort of familial ALS, where one subgroup had reduced survival compared to the second subgroup and also had a higher proportion of cognitive impairment, in particular ALS-FTD (see figure 5.10).
A. Familial subgroups identified in chapter four

This emphasizes the importance of using registry data to identify subgroups of ALS patients that may be associated with an unknown gene.

Familial ALS patients, with better survival outcomes, may carry a single, as yet unknown, gene. The longevity noted in FALS cases without the C9orf72 repeat expansion may be due to a pathological gene with a less deleterious effect on the phenotype.

A number of trinucleotide repeat disorders have been associated with neurological disease. Features of the trinucleotide repeat disorders include anticipation from generation to generation due to expansion instability, and the notion that fecundity may be impaired because of genetic expansions. These factors will need to be addressed in ALS patients with the C9orf72 repeat expansion.

Data presented here would suggest that routine testing of all patients with ALS for the presence of the repeat expansion is not indicated. Patients with a negative family history of ALS and FTD, who have normal cognition and behaviour, are extremely unlikely to harbour the repeat expansion.
Testing for the presence of the repeat expansion in the "at risk" group (patients with evidence of cognitive and behavioural impairment and a family history of ALS or FTD) outside of a research setting should be undertaken with caution. Diagnostic testing demands a higher degree of certainty, and because of the extensive implications both for patients and their family members, stringent quality control is required. At present, the precise cut-off for a pathogenic expanded number of repeats remains unclear, and there is a degree of variability in the maximum repeat number identified in control populations. The maximum size of the pathological expansions cannot be determined using repeat prime PCR and formal diagnostics will require definitive validation using Southern blotting.

Whether pre-symptomatic family members should be offered testing is also unclear. At present, there are insufficient data to predict the probability of an asymptomatic person with the repeat expansion developing disease, however further work on this topic will be presented in the next chapter. While pre-symptomatic testing would be of value in a research setting, underpinned by strict ethical guidelines, a larger body of research will be required to sufficiently address important issues such as the degree of penetrance, the probability of an expansion in repeat number from one generation to the next, and patterns of inheritance. These issues are investigated in the following chapter of family aggregation in ALS.

This study is limited by the relative small size of the cohort with the repeat expansion (n=21), although the larger size of the unaffected cohort (n=170) provides statistical power. Detailed cognitive and family history studies are time consuming both for the investigator and the patient, and attrition rates are high in longitudinal studies. This study has provided strong evidence that repeat expansion is associated with a characteristic cognitive and behavioural phenotype, and shorter survival.

5.5.1 Conclusion

In summary, ALS patients with an expanded hexanucleotide repeat in C9orf72 represent a recognizable subphenotype characterised by a lower age of onset, the presence of cognitive and behavioural impairment, specific neuroimaging changes, a strong family history of
neurodegeneration, and reduced survival. The repeat expansion was not present in true sporadic ALS patients with no behavioural abnormalities.

In order to determine if there is aggregation of other neurodegenerative and psychiatric conditions in relatives of patients with the C9orf72 repeat expansion, to quantify the risk to other family members of a patient with the C9orf72 repeat expansion of developing ALS, and to investigate the penetrance of the C9orf72 repeat expansion, a large family aggregation study is warranted. Such a study is described in the next chapter.

These findings demonstrate that detailed phenotyping and careful characterization within a population-based cohort can rapidly generate useful clinical algorithms in the context of novel genetic discoveries.
Chapter 6  Family Aggregation Study

6.1 Introduction
The previous chapter described the phenotype associated with the \textit{C9orf72} repeat expansion. Many questions regarding this gene are outstanding, including: whether aggregation of other neurodegenerative conditions and psychiatric conditions occur in families of ALS patients carrying the repeat expansion, what is the risk of family members of ALS patients with the repeat expansion of developing ALS compared to the risk in relatives of ALS patients not carrying the repeat expansion, and how penetrant is the gene?
A thorough family aggregation study could also seek to determine the true rate of familial ALS, which as demonstrated in the meta-analysis in chapter 4 (4.2) is currently 5.1%.

6.2 Aims
The primary aim of this study is to carry out a comprehensive Family Aggregation Study, comparing family members of patients with ALS to family members of controls. This will identify conditions that occur to a higher degree among family members of people with ALS compared to relatives of controls. Such a method can apportion risk to other family members and may also inform future genetic studies.

\textbf{Aggregation of ALS:} The true rate of familial ALS is unknown. The risk to relatives of ALS patients, of themselves developing the disease, has not been estimated for the presence or absence of the \textit{C9orf72} repeat expansion in the ALS patient
Specific aims:

1. To determine the true rate of familial ALS in a population-based study
2. To determine the risk to relatives of ALS patients of developing ALS, and to stratify this risk according to the absence or presence of the \textit{C9orf72} repeat expansion
3. To use information collected to direct future genetic studies
To achieve these aims, incident ALS patients from the four-year period 2008-2011 were identified using the Irish ALS register and detailed family history taking techniques and genealogical methods were used to identify all cases of ALS in the families of a representative portion of incident ALS patients. The same data was collected from matched controls. Comparisons are made between cases and controls including family history, epidemiological data, family structure and size, and fecundity. Large pedigrees, negative for known genes are considered for future genetic studies.

**Aggregation of neurodegenerative and psychiatric disease:** The rate of aggregation of neurodegenerative diseases among relatives of patients with ALS has been reported in seven previous family aggregation studies, however none of these studies segregate for the presence or absence of the C9orf72 repeat expansion. Although case studies exist for the aggregation of psychiatric disease in the families of ALS patients, no family aggregation studies exist looking at this.

**Specific aims:**

1. To determine familial recurrence risk ($\lambda_a$) for neurodegenerative diseases (Parkinson disease, dementia) and neuropsychiatric diseases (depression, schizophrenia), and suicide among relatives of ALS patients, and to stratify this risk according to the absence or presence of the C9orf72 repeat expansion

These aims were achieved through a family aggregation study.

**6.3 Methods**

**6.3.1 Study design**

In the **Family History Method**, the proband answers questions about the health of their relatives[173]. An abbreviated family history may be taken by enquiring about the presence of specific diseases in relatives, without clarifying further details of the illness or demographic factors. A detailed family history, where the researcher collects as much information on as many family members as possible, including specifics regarding any disorders identified (e.g. age at onset etc), demographic details, and information necessary to
verify medical conditions reported (e.g. date of death for death certificate verification), may also be taken. Clarification of the history from more than one family member is advisable, particularly if the proband has evidence of cognitive impairment. This method makes possible the collection of information on large numbers of relatives. Provided histories are properly verified using independent sources (e.g. death certificates, hospital records), this is a very reliable method for collecting family aggregation data.

The Family Study Method differs from the family history method in that, in this case, each member of the proband's family is contacted directly to enquire about health status[173]. This method ensures that the information collected is accurate as each person reports his or her own medical history directly to the researcher. If features of the condition under study (e.g. tremor in a study of aggregation of PD) are reported then the researcher arranges to visit the person and verifies the diagnosis by clinical examination. This method is very labour intensive and expensive to perform compared with the family history method. Patients may also be reluctant to enter into a study if every living member of their family is to be contacted.

A case-control study design was chosen for this family aggregation study. The availability of a population based ALS Register permitted case selection for patients diagnosed in the four-year period 2008-2011. A detailed family history approach was used to collect data on all first- and second-degree relatives of cases and age- and sex-matched controls. Deaths were validated by death certification where possible. Ideally a family study method would have been used but factors such as cost, study-length, manpower, and ethical restrictions make this impossible. As the study focused on patients with ALS, which has a short survival time, the family study method would not yield the volume of information that a detailed family history study (with careful death certificate verification) would over the same time period. Recruitment, data collection and subsequent analysis were carried out from July 2009 – June 2012. A pilot study was performed initially to assess the practical application of the questionnaire.
6.3.2 Power

Power for the family aggregation study was calculated using a method described by Betensky and colleagues[213]. The generalized likelihood ratio test is applied assuming: γ is the log odds ratio for disease in one family member given disease status of another family member (γ = 0.3), δ is the log-odds of disease in a family member conditional on no disease in the remaining family members (δ = -2), family size (n) is fixed at five non-probands, and that the sample of families contains an equal number of case and control families (i.e. r = 0.5). A sample of 200 families (100 cases and 100 controls) would have a power of 0.80 under these conditions. Calculations were undertaken using the printed table as the freeware calculator is currently under maintenance, and therefore the family size is set at 5 (personal correspondence with author, Professor Betensky).

6.3.3 Data collection

As described previously all Irish patients with ALS are included in the Irish ALS Register, which has complete case ascertainment for the Republic of Ireland since 1996. New cases are identified by the Irish Register on an ongoing basis. A representative portion of ALS patients (target 50%) diagnosed in the four-year period were approached to take part in the study. These patients were selected randomly from new cases registered.

6.3.4 Design of questionnaire

A 22-page family history self-administration questionnaire was made available by the US National Institute of Health (see appendix B). This questionnaire collects information on first-, second-, and third-degree relatives (first-degree: children, parents, siblings, second-degree: nieces/nephews, aunts/uncles, grandparents, and third-degree: first cousins.). The information collected on each person includes age, vital status, sex, place of birth, date of death, and place of death. A general question was also asked inquiring about the possibility of more distant relatives, not specifically mentioned on the questionnaire, having ALS.
SECTION III. PROBAND’S PARENTS

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Birthdate</th>
<th>Date/Place/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What is the racial background of your Mother? ________________________________

What is the racial background of your Father? ________________________________

What was your mother's place of death? ________________________________

What was your father's place of death? ________________________________

What is the address of your parent(s) if living? ________________________________

Did/did either of these people have any health problems? ________________________________

Did/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc? ________________________________

Explain ____________________________________________________________________________

Figure 6.1 Section from the pilot family history questionnaire

Pilot study for questionnaire: In 2009 a pilot study was carried out using the 22-page questionnaire (Appendix B). It was sent to 40 patients who had been diagnosed with ALS prior to 2008 and they were asked to complete it. 30 days after the questionnaire had been sent only 47.5% (19) of patients had returned it. All patients were contacted to ask them for their feedback on the form:

'Too long....so many pages'

'Couldn't understand the instructions'

'Too time-consuming'

'Very difficult to complete'

'Took hours to complete!'

'I would rather answer the questions over the telephone'

A decision was made to reformat the questionnaire. It was made significantly shorter using a guide to survey design by Dillman[214].

The following changes to the questionnaire were made:

177
- Reduced in length from 22 pages to 13 pages
- New shorter questionnaire was printed back and front, thus reducing the volume of paper from 22 pages to seven pages
- The language was simplified and superfluous text was omitted. The font was changed to a sans serif font for ease of reading.
- A logo, specifically designed for the project by the author, was included to make the questionnaire more appealing to complete

![Family aggregation study logo](image.png)

*Figure 6.2 Family aggregation study logo: Family tree with fingerprint leaves and DNA tree trunk*

From the pilot questionnaire study it was also noted that cognitive impairment could be inferred very accurately by the amount of difficulty that the respondent encountered completing the form. Completed forms from the pilot study were graded as i) form completed in full, ii) form almost completed but minor gaps in information, and iii) form incomplete with lots of missing information. Patients with normal cognition completed the form in full in 64% of cases, and almost completely in 36% of cases, compared to 28%, and 36% respectively in patients with cognitive impairment (p=0.03). Patients with cognitive impairment were unable to understand the instructions and put incorrect information into the space provided for answers, as well as paucity of information on other family members. This highlighted the importance of having a second family member help the patient with the family history information if the patient was cognitively impaired.
Consideration was given to the use of an online questionnaire (e.g. SurveyMonkey®), as it is cheap, the link can easily be sent to large groups, data is exportable to SPSS, there are no printing or postage costs, and results are returned more quickly than in the paper format. An online survey was decided against as confidential information relating to medical health and personal information would be collected and the confidentiality of online data could not be guaranteed. There were also concerns regarding executive function and the ability to complete an online form, as well as computer literacy.

The questionnaire used in the full study is in Appendix C.

6.3.5 Inclusion and exclusion criteria for recruitment and selection of participants

ALS Patients: The study was open to all patients with a diagnosis of definite, probable, or possible ALS by El Escorial criteria, on the ALS Register in Ireland diagnosed between January 1st 2008 and December 31st 2011. Patients were excluded if the diagnostic inclusion criteria for ALS were not met or the patient did not qualify for inclusion on the ALS Register.

The aim was to collect data on cases diagnosed within the period of the study.

Controls: For each patient, an age- (+/- 1 year) and sex-matched control was collected. Control participants were recruited through a network that included a general newspaper advertisement campaign, which did not mention the focus of the study, and primary care providers.

Patients and controls willing to participate were consented using standard consent methods.

6.3.6 Family history data collection

Initial contact: All patients identified by the Irish ALS Register have previously consented to the inclusion of their details on an electronic database for research purposes. Patients constituting a representative portion of ALS patients diagnosed within the study period (or the closest living relative or spouse if the patient was unable to speak on the telephone) were contacted outlining the nature of the study in non-scientific language.

Telephone call #1: Patients willing to participate in research were initially contacted by telephone. The purpose of the initial telephone was to explain the purpose of the research
and the nature of the information being collected. Information and consent were not collected at this time, but rather the participating individual was informed that an information leaflet/consent form and family history questionnaire would be mailed to their home.

**Questionnaire:** Immediately after this telephone call, the consent form and family history questionnaire were mailed in a stamped-addressed envelope for ease of postal return. The participant was encouraged to seek help from at least one other close relative.

The questionnaire asks participants about medical conditions in all first, second and third degree relatives. The following information was also collected for each family member:

- Age at time of form of completion or death
- Full name
- Place and cause of death if deceased

Data collection primarily concentrated on the participating individual and his/her siblings, parents, grandparents, maternal and paternal uncles and aunts and cousins. Medical data was not collected on children less than 18 years of age unless deceased, however demographic data was collected on all live offspring (including those less than 18 years) of patients and controls.

**Telephone call #2:** Within seven days of receipt of the completed questionnaire and consent form, we contacted the participant by telephone to clarify information completed in the family history questionnaire.

To increase validity of the family history data and power of the study, information contained in the completed questionnaire was verified by at least one other relative within each family in the process of completion of the family history questionnaire, particularly if the patient was cognitively impaired.

6.3.7 **Procedures for processing the family history questionnaire**

Once the participant returned the questionnaire, a follow-up phone call/visit was made to all participants to further clarify family history information.

**History of Neurodegeneration:** Where a family history was positive for any neurodegenerative condition, further questioning sought to verify the diagnosis. During the
telephone interview the following open-ended question was asked: “Have you or any family member ever had any neurological disease?” In order to establish or clarify a diagnosis of a neurodegenerative disease the following semi-structured interviews were undertaken where appropriate.

**Dementia:**
- Diagnosed by clinician?
- Predominantly amnestic or behavioural/personality change?
- Age at onset?
- Severity - Able to live independently (mild) or requiring supervised housing (moderate/severe)?

**Parkinson’s disease:**
- Diagnosed by clinician?
- Bradykinesia, rigidity, tremor?
- Unilateral at beginning?
- Not related to other medications?
- Any related dementia?
- Age at onset?

**Multiple Sclerosis:**
- Diagnosed by clinician?
- MRI?
- Period of diagnosis?
- Age at onset?
- Symptoms and survival?

**History of Neuropsychiatric disease:** A family history of psychiatric disorders was ascertained and verified using a combination of information from the questionnaire and a structured telephone interview. During the telephone interview after completion of the questionnaire, the following open-ended question was also asked: “Have you or any family member ever had any psychiatric disorder or spent time in a psychiatric hospital?” In order to establish and clarify a diagnosis of a psychiatric disease the following semi-structured interview was completed where appropriate.

**Depression:**
- Diagnosed by clinician?
- Severity of depression - mild/moderate or pervasive and requiring hospitalisation/ECT?
- Any episodes of mania?
- Diagnosis of bipolar/manic depression?

**Schizophrenia:**
- Diagnosed by clinician?
- Paranoid delusional disorder requiring psychiatric intervention?
- Age at onset?
- Symptoms?

A condition was classified as an unspecified psychiatric disorder when a relative of the proband has a psychiatric disorder but the proband was unaware of a definitive psychiatric diagnosis. Attempts made to discover if the psychiatric disorder was predominantly a disturbance of mood or predominantly characterised by psychotic features. These cases were not included in analysis.

**Clarification of names, places of death and dates of death:** Accurate information regarding name, place and date of death, of participants and family members, helped to identify death certificates. Many people were unaware of the exact age and year in which a relative from a previous generation died. Information such as approximate age of deceased at death and the approximate year/decade of death was collected. It was helpful to ask the participant what age they were when their relative (e.g. grandmother) died and then calculate the approximate year that way. The place/townland of death was also requested.

Upon completion of the phone call, the participant and their family have now given the researcher as much information as they have available to them. If a family member offered to check gravestones or parish records they were contacted again for a further telephone call at a set interval.

**6.3.8 Online resources for verifying family history**

**Online Irish Census:** In order to obtain as much information as possible on every kindred a search was made of the online Irish censuses from 1901 and 1911.
This is an extremely useful aid in estimating the age at death. The online census is also helpful in identifying the townland/address for individuals who died near the time of the census. All of this information is necessary to obtain a death certificate.

**Family Search:** ([www.familysearch.org](http://www.familysearch.org)) is an online resource provided by a religious organization which maintains records for billions of people throughout the world. It has the dates of death and birth for many Irish people for the past century.

### 6.3.9 Verification of reported cause of death with official death certificates

All Irish birth, death and marriage records are available to the public through the General Registries Office (GRO) research room on Lower Liffey Street, Dublin 1. Attempts were made to get the death certificate for every family member who died in the island of Ireland during the period 1864-2012. Death certificates from prior to 1864 are not available in the GRO and must be requested from Parish records.

**Manual search:** A reference book for each year, split into quarters (e.g. 1951 January-March), is searched for the name of the deceased. A reference number for the deceased is then used to acquire the death certificate.

**Computer-assisted search:** As of January 2011 the research group was granted permission to use the research computer in the GRO. This method of searching was much faster than the manual search method and allowed a much larger number of certificates to be collected. The
first name and surname of the deceased were entered into a search engine, along with county of residence and time period to be searched. For frequently occurring names (e.g Patrick Murphy) it was essential to have details such as if they were single/married or widowed at the time of death as well as the townland/address at which they resided at the time of death. When the death certificate was obtained the reported cause of death was compared to the cause of death on the death certificate. Note was taken of whether the death certificate added addition information (e.g dementia) or failed to verify a cause of death reported by family members.

For many deceased relatives the cause of death was unknown and the death certificate added valuable information to the family history.

6.3.10 Database

A database was created in SPSS to record data on all of the kindreds included in the study. Each family was given a unique code. The database was split into two files; the first file contained information on the proband and their family structure (104 variables), and the second file contained information on every relative within a family (105 variables). For each relative age, sex, and relationship to proband was recorded. If the relative was living any co-morbidities were noted. If the person was deceased then the cause of death was recorded. If the death was verified by death certification then the official cause of death was recorded. Attempts were made to quantify the concordance between reported cause of death by family members and death certification and vice versa.

In many cases the proband had no information on the cause of death of family members from past generations. Attempts were made to quantify the additional information added by the use of death certification.

6.3.11 Data storage and confidentiality

All of the information obtained from participating families was stored on a software-encrypted password-protected database (TrueCrypt®). Each individual included in the database was assigned a unique numeric identifier to further ensure anonymity.
6.3.12 Statistical analysis

Baseline characteristics are tested for difference using the Chi square test for independence and the independent sample t test. Non-parametric tests are used if the distribution is not normal.

Relative risk of the disease in question, lambda (λ), was calculated by dividing the rate of disease among relatives of patients with ALS by the rate of disease among relatives of controls (see section 2.1.5). Cumulative incidence estimates calculate the risk to a relative of an ALS patient manifesting the condition by a particular age when compared with the relative of a control. These results are represented graphically on a cumulative incidence 'hazard rate' plot, which is the inverse of a Kaplan-Meier plot. Cox proportional hazards analysis is used to calculate the Hazard rate ratio and 95% confidence interval for each disorder in relatives of ALS patients compared to relatives of controls, after weighting for proportion of relative type (e.g. sibling, aunt, etc) and correcting for other factors such as degree of relatedness, sex etc.

The relative risk and hazard rate are calculated for:

- Rate of ALS in relatives of ALS patients compared to controls
- Subgroup analysis looking at risk of ALS in relatives of ALS patients with the C9orf72 repeat expansion and those without the expansion
- Rate of neurodegenerative and psychiatric conditions in relatives of ALS patients compared to the rate among relatives of controls
- Subgroup analysis looking at risk of neurodegenerative and psychiatric conditions in relatives of ALS patients with the C9orf72 repeat expansion, and those without the expansion
- Subgroup analysis looking at rates of dementia and ALS in relatives of cognitively impaired ALS patients compared to relatives of cognitively normal ALS patients without the C9orf72 repeat expansion

Cluster analysis included direct observations of histograms showing the frequency in number of affected relatives between cases and controls. Binary logistic regression in a generalized
linear model was used to assess for clustering within kindreds. The advantage of this method is that family size and number of affected individuals within kindreds were taken into account.

**Segregation analysis:** was carried out on the pedigrees of familial ALS cases to calculate penetrance estimates. Penetrance was estimated using two methods: i) manual method, and ii) online penetrance estimate calculator (PenCalcWeb®[215]).

The manual method for calculating segregation ratio and penetrance estimate, described by Lazzarini and colleagues[216], is estimated using the following equations:

\[
\text{Segregation ratio, } S_R = \frac{\text{Number of affected individuals in a generation}}{\text{Total number of individuals in the same generation}}
\]

\[
\text{Penetrance, } P = 2 \times S_R
\]

*Equation 3 Equations for manual calculation of segregation ratio and penetrance estimate*

Comparisons were made between the two methods. Penetrance of ALS was estimated from generation to generation, in families with more than one member with ALS, with and without evidence of the \( C9orf72 \) repeat expansion. Penetrance was also calculated for the transmission of dementia, psychiatric conditions and ALS in kindreds with the \( C9orf72 \) repeat expansion.

Statistical analysis was carried out using SPSS 16. All statistical testing was performed at the conventional 2-tailed \( \alpha \) level of 0.05. Where multiple comparisons were made the Bonferroni correction was utilised.
6.4 Results of Aggregation study: General

6.4.1 Recruitment of ALS patients

In the four-year study period from 2008 to 2011, 366 people in the Republic of Ireland were diagnosed with definite, probable, or possible ALS by El Escorial criteria.

![Figure 6.4 Recruitment for the Family Aggregation Study](image)

A representative sample of the patients diagnosed within the study period, 2008 to 2011, were approached to take part in the family history study. The 219 people approached for inclusion in the study were a representative sample of the cohort (table 6.1), and there were no differences between those contacted for recruitment and those not contacted for recruitment, in terms of age of onset or diagnosis, sex, El Escorial criteria at first assessment, site of onset, or the proportion reporting a positive family history,
<table>
<thead>
<tr>
<th></th>
<th>Contacted for recruitment n=219</th>
<th>Not contacted for recruitment n=147</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age onset</td>
<td>64.4 years (SD 11.1)</td>
<td>64.3 years (SD 11.9)</td>
<td>0.98</td>
</tr>
<tr>
<td>Age diagnosis</td>
<td>65.2 years (SD 11.3)</td>
<td>65.5 years (SD 11.9)</td>
<td>0.82</td>
</tr>
<tr>
<td>Sex</td>
<td>103 female (47%)</td>
<td>60 female (41%)</td>
<td>0.29</td>
</tr>
<tr>
<td>El Escorial</td>
<td>Definite 147 (67%)</td>
<td>Definite 108 (73%)</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Probable 30 (13.6%)</td>
<td>Probable 17 (11.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Possible 42 (19.2%)</td>
<td>Possible 20 (13.6%)</td>
<td></td>
</tr>
<tr>
<td>Site onset</td>
<td>Bulbar 79 (36%)</td>
<td>Bulbar 56 (38%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Limb 129 (58%)</td>
<td>Limb 83 (56.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other 11 (5%)</td>
<td>Other 8 (5.5%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Family history ALS</td>
<td>18 cases FALS (8.2%)</td>
<td>12 cases FALS (8.1%)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Table 6.1 Baseline characteristics of the representative sample contacted (n=219) compared to the sample not contacted (n=147).

Of 219 ALS patients contacted, 212 agreed to be included in the study and seven declined.

Family history questionnaires were sent to 212 ALS patients. The response rate was 81%.

The most common reason for non-completion of a family history questionnaire was death of participant prior to return of the form (36/40). Table 6.2 compares baseline characteristics between those who completed a family history questionnaire and those who did not.
<table>
<thead>
<tr>
<th>Completed participation in study (n=172 patients, 169 individual families)</th>
<th>Did not complete participation in study n=47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age onset</td>
<td>63.1 years (SD 11.4)</td>
</tr>
<tr>
<td>Age diagnosis</td>
<td>63.9 years (SD 11.6)</td>
</tr>
<tr>
<td>Sex</td>
<td>78 female (45%)</td>
</tr>
<tr>
<td>El Escorial</td>
<td>Definite 106 (61.6%)</td>
</tr>
<tr>
<td>Probable 25 (14.5%)</td>
<td>Probable 5 (10.5%)</td>
</tr>
<tr>
<td>Possible 34 (19.8%)</td>
<td>Possible 8 (17%)</td>
</tr>
<tr>
<td>Site onset</td>
<td>Bulbar 59 (34.3%)</td>
</tr>
<tr>
<td>Limb 103 (60%)</td>
<td>Limb 26 (55.5%)</td>
</tr>
<tr>
<td>Other 10 (5.7%)</td>
<td>Other 1 (2%)</td>
</tr>
<tr>
<td>Family history ALS</td>
<td>13 cases FALS (7.7%)</td>
</tr>
</tbody>
</table>

Table 6.2 Demographic details of those patients who returned their family history questionnaire versus those who did not return a questionnaire or declined to participate in the study

The patients who declined to participate (n=7) and who did not return a questionnaire (n=40) were significantly older at symptom onset and diagnosis, and were more likely to have definite disease at initial presentation. The majority of non-responders died before completion of the questionnaire and as a result there was a significant difference between time from diagnosis to death in the group who did respond compared to those who did not (median survival 22 months in responders versus 13 months in non-responders, p<0.0001).

6.4.2 Recruitment of controls

Controls were matched for age (within one year) and sex. 311 controls were contacted. The response rate was 61.7% (192/311).
6.4.3 Quality of data collected

Family histories were graded as having complete data capture, good data capture, incomplete data capture, or poor data capture (table 6.3). Data collection from controls yielded higher rates of complete data collection and lower rates of incomplete data collection ($X^2=18.0$, $p<0.0001$). Disability and fatigue among patients and their caregivers was the main reason cited for recording histories with fewer third degree relatives.

<table>
<thead>
<tr>
<th>Quality of Data Capture</th>
<th>Case n=172</th>
<th>Control n=192</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete data capture</td>
<td>70 (40.7%)</td>
<td>101 (52.6%)</td>
</tr>
<tr>
<td>(all 1st, 2nd degree relatives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good data capture</td>
<td>61 (35.5%)</td>
<td>75 (39.1%)</td>
</tr>
<tr>
<td>(most 1st, 2nd degree relatives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data capture incomplete</td>
<td>38 (22.1%)</td>
<td>16 (8.3%)</td>
</tr>
<tr>
<td>(have 1st degree and some 2nd degree relatives)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor data capture</td>
<td>3 (1.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>(data on 1st degree relatives only)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6.3 Comparison of family histories collected between cases and controls

6.4.4 Demographic comparison between cases and controls

Information on 172 ALS patients (from 169 families) and 192 controls was collected. 44% (79/172) of the cases were female, compared with 47% (93/192) of the controls ($p=0.83$). Patients and controls were well matched with a mean age of 63.9 years (SD 11.8 years) in patients and 63.5 years (SD 10.2 years) in controls at the time of family history data collection ($p=0.42$). Female cases had a mean age of 65.3 years (SD 11.3 years) compared to 64.2 years (SD 9.5 years) in controls ($p=0.49$). Males were similarly matched with a mean age of 62.7 years (SD 12.2 years) in male cases and 62.9 years (SD 10.7) in male controls ($p=0.94$).
6.4.5 Number of first- and second-degree relatives

Information was available on 4,376 first- and second-degree relatives of 172 cases (in 169 pedigrees) and 6,030 first- and second-degree relatives of 192 controls (192 pedigrees). 326 relatives of cases and 396 relatives of controls were excluded from analysis as the only available information was the gender. Analysis was carried out on 4,050 first- and second-degree relatives of cases and 5,634 first- and second-degree relatives of controls. The breakdown of number of each category of relative is presented in table 6.4.

<table>
<thead>
<tr>
<th></th>
<th>Relatives of</th>
<th>Relatives of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cases</td>
<td>controls</td>
</tr>
<tr>
<td><strong>1st degree relatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Offspring</td>
<td>409</td>
<td>525</td>
</tr>
<tr>
<td>Siblings</td>
<td>736</td>
<td>808</td>
</tr>
<tr>
<td>Parents</td>
<td>334</td>
<td>382</td>
</tr>
<tr>
<td><strong>2nd degree relatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aunt</td>
<td>454</td>
<td>767</td>
</tr>
<tr>
<td>Uncle</td>
<td>447</td>
<td>782</td>
</tr>
<tr>
<td>Grandmother</td>
<td>205</td>
<td>342</td>
</tr>
<tr>
<td>Grandfather</td>
<td>196</td>
<td>332</td>
</tr>
<tr>
<td>Niece/Nephew</td>
<td>1269</td>
<td>1696</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4050</td>
<td>5634</td>
</tr>
</tbody>
</table>

Table 6.4 Breakdown of relative clarification for relatives of cases and controls

The mean age of each subgroup of relative is compared between cases and controls in table 6.5.
Table 6.5 Comparison of mean ages for different categories of relative between cases and controls

6.4.6 Cause of death

Forty per cent (1640) of relatives of cases were deceased compared to 43% (2429) of relatives of controls (p=0.006). Cause of death was split into eight categories as follows: cardiac, neurological, respiratory, gastroenterology, cancer, stroke, other, and unknown (see table 6.6). A table describing death by specific cause is available in Appendix D.

<table>
<thead>
<tr>
<th>Category</th>
<th>Relative of Case n = 1606</th>
<th>Relative of Control n = 2416</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>Count 425</td>
<td>Count 650</td>
</tr>
<tr>
<td></td>
<td>% within group 26.5%</td>
<td>% within group 26.9%</td>
</tr>
<tr>
<td>Neurology</td>
<td>Count 117</td>
<td>Count 108</td>
</tr>
<tr>
<td></td>
<td>% within group 7.3%</td>
<td>% within group 4.5%</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Count 156</td>
<td>Count 272</td>
</tr>
<tr>
<td></td>
<td>% within group 9.7%</td>
<td>% within group 11.3%</td>
</tr>
<tr>
<td>Gastro</td>
<td>Count 11</td>
<td>Count 54</td>
</tr>
<tr>
<td></td>
<td>% within group 0.7%</td>
<td>% within group 2.2%</td>
</tr>
<tr>
<td>Cancer</td>
<td>Count 342</td>
<td>Count 442</td>
</tr>
<tr>
<td></td>
<td>% within group 21.3%</td>
<td>% within group 18.3%</td>
</tr>
<tr>
<td>Other</td>
<td>Count 338</td>
<td>Count 486</td>
</tr>
<tr>
<td></td>
<td>% within group 21.0%</td>
<td>% within group 20.1%</td>
</tr>
<tr>
<td>Stroke</td>
<td>Count 114</td>
<td>Count 202</td>
</tr>
<tr>
<td></td>
<td>% within group 7.1%</td>
<td>% within group 8.4%</td>
</tr>
<tr>
<td>Unknown</td>
<td>Count 103</td>
<td>Count 202</td>
</tr>
<tr>
<td></td>
<td>% within group 6.4%</td>
<td>% within group 8.4%</td>
</tr>
<tr>
<td>Total</td>
<td>Count 1606</td>
<td>Count 2416</td>
</tr>
<tr>
<td></td>
<td>% within group 100.0%</td>
<td>% within group 100.0%</td>
</tr>
</tbody>
</table>

Table 6.6 Cause of death by general category

The rate of cardiac causes of death are equal in both groups; 26.5% compared to 26.9%. Within the category of cardiac causes, the rates of myocardial infarction (MI) are also equal:
15.8% (252 relatives of cases died of MI), compared to 15.9% (386 relatives of controls died of MI) (see Appendix D). The death due to cancer is also higher among relatives of ALS patients compared to controls (21.3%, compared to 18.3%).

6.4.7 Verification of cause of death

Verification of the cause of death with official death certification was possible in 57% of deceased relatives of cases (934/1,640) and 63% of deceased relatives of controls (1,560/2,429), p<0.0001.

Details of the reported cause of death, and the certified cause of death were available for 900 deceased relatives of cases, and 1,555 deceased relatives of controls (table 6.7).

<table>
<thead>
<tr>
<th></th>
<th>Relative of Case n = 900</th>
<th>Relative of Control n = 1555</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>425</td>
<td>779</td>
</tr>
<tr>
<td>% within group</td>
<td>47.2%</td>
<td>50.1%</td>
</tr>
<tr>
<td><strong>No</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>137</td>
<td>173</td>
</tr>
<tr>
<td>% within group</td>
<td>15.2%</td>
<td>11.1%</td>
</tr>
<tr>
<td><strong>NIA so cert added information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>296</td>
<td>534</td>
</tr>
<tr>
<td>% within group</td>
<td>32.9%</td>
<td>34.3%</td>
</tr>
<tr>
<td><strong>Cert did not include reported psych or neuro info</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>42</td>
<td>54</td>
</tr>
<tr>
<td>% within group</td>
<td>4.7%</td>
<td>4.4%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Count</td>
<td>900</td>
<td>1555</td>
</tr>
<tr>
<td>% within group</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 6.7 Compares the numbers of death certificates which match the reported case of death (Yes), those that do not match (No), and those that add a cause of death that was previously unavailable (No information available [NIA] so cert added information), and those certificates omitting a reported neurological or psychiatric cause of death. $X^2=9.0$ p=0.032

Reported cause of death matched the certified cause of death in 1204 of 1514 reported cases. Death certificates added a cause of death in 830 relatives where a cause of death was unknown. Death certification added a previously unreported neurological cause of death in 165 certified deaths (79 in relatives of cases and 86 in relatives of controls).
6.5 Results of Aggregation study: Familial ALS

6.5.1 Family aggregation study: Reported cases of familial ALS at study inception

7.7% (13/169) of patients with ALS included in the study originally reported a family history of ALS to the register when initially diagnosed with ALS (details of these 13 families in table 6.8).
<table>
<thead>
<tr>
<th>Reported</th>
<th>Relative</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Confirmation</th>
<th>Genetics</th>
<th>FALS definition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td>Proband</td>
<td>60 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>60 yrs</td>
<td>ALS</td>
<td>Death Certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>41 yrs</td>
<td>ALS</td>
<td>Medical notes and death certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 2</td>
<td>Proband</td>
<td>53 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>No known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>75 yrs</td>
<td>ALS</td>
<td>ALS reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>N/A</td>
<td>ALS</td>
<td>ALS reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4th degree</td>
<td>60 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5th degree</td>
<td>64 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 3</td>
<td>Proband</td>
<td>56 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>55 yrs</td>
<td>ALS</td>
<td>Medical notes and death certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>84 yrs</td>
<td>ALS/PLS</td>
<td>ALS not reported</td>
<td></td>
<td>Death certificate added diagnosis</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>60 yrs</td>
<td>ALS</td>
<td>ALS not reported</td>
<td></td>
<td>Death certificate added diagnosis (MND)</td>
</tr>
<tr>
<td>Family 4</td>
<td>Proband</td>
<td>60 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>63 yrs</td>
<td>ALS</td>
<td>Medical notes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*as per proposed criteria for FALS in section 3.1.6†
<table>
<thead>
<tr>
<th>Reported</th>
<th>Relative</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Confirmation</th>
<th>Genetics</th>
<th>FALS definition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 5</td>
<td>1st degree</td>
<td>77 yrs</td>
<td>ALS</td>
<td>Suspicious history, confirmed with medical notes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>65 yrs</td>
<td>ALS</td>
<td>Suspicious history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 6</td>
<td>1st degree</td>
<td>58 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>72 yrs</td>
<td>ALS</td>
<td>ALS reported in history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 7</td>
<td>1st degree</td>
<td>64 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>50 yrs</td>
<td>ALS</td>
<td>ALS reported in history, Death certificate (MND x 2 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>79 yrs</td>
<td>ALS</td>
<td>ALS reported in history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 8</td>
<td>2nd degree</td>
<td>85 yrs</td>
<td>ALS</td>
<td>ALS reported in history, Death certificate did not confirm diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 9</td>
<td>Proband</td>
<td>85 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Not tested</td>
<td>Probable FALS</td>
</tr>
<tr>
<td>Reported</td>
<td>Relative</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Confirmation</td>
<td>Genetics</td>
<td>FALS definition*</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>------</td>
<td>-----------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td>------------------</td>
</tr>
<tr>
<td>1st degree</td>
<td></td>
<td>55 yrs</td>
<td>ALS</td>
<td>Medical notes, Death certificate</td>
<td>No known gene</td>
<td>Possible FALS</td>
</tr>
<tr>
<td><strong>Family 10</strong></td>
<td>Proband</td>
<td>67 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td>No known gene</td>
<td>Possible FALS</td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>61 yrs</td>
<td>ALS</td>
<td>History suspicious, medical notes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5th degree</td>
<td>70 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5th degree</td>
<td>80 yrs</td>
<td>ALS</td>
<td>Medical notes, Death certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family 11</strong></td>
<td>Proband</td>
<td>61 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td>No known gene</td>
<td>Possible FALS</td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>68 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family 12</strong></td>
<td>Proband</td>
<td>57 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td>No known gene</td>
<td>Possible FALS</td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>57 yrs</td>
<td>ALS</td>
<td>Medical notes, Death certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>N/A</td>
<td>ALS</td>
<td>Medical report</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Family 13</strong></td>
<td>Proband</td>
<td>63 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td>No known gene</td>
<td>Suspected FALS</td>
</tr>
<tr>
<td></td>
<td>4th degree</td>
<td>36 yrs</td>
<td>ALS</td>
<td>Examinated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
We found that one of the most sensitive screening questions for determining if another family member had been affected by ALS was 'Has anyone else in your family ever had something similar to the condition that you have?'

6.5.2 Family aggregation study: Additional relatives found to have ALS through aggregation study

Through the family aggregation study it was discovered that an additional 14 'sporadic' ALS cases from different families had a family history of ALS (table 6.9). Four of these were ascertained through death certification when there was a suspicious history of progressive immobility or speech problems with rapid deterioration to death, seven were ascertained through death certification where the patient did not know the cause of death or reported a different cause of death, one was ascertained from a review of medical notes where there had been a suspicious history in a relative, and two were ascertained from detailed family history linking affected individuals from apparently sporadic kindreds.
<table>
<thead>
<tr>
<th>Not reported</th>
<th>Relative</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Confirmation</th>
<th>Genetics</th>
<th>FALS definition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proband</td>
<td>66 yrs</td>
<td>ALS</td>
<td></td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td>Family 1</td>
<td>1st degree</td>
<td>70 yrs</td>
<td>ALS</td>
<td>Suspicious history reported of rapidly progressive immobility with dementia;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death certificate (death by respiratory failure)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>65 yrs</td>
<td>ALS</td>
<td>Suspicious history reported of slurred speech and immobility over 2 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proband</td>
<td>57 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td>Family 2</td>
<td>1st degree</td>
<td>47 yrs</td>
<td>ALS</td>
<td>Suspicious history of progressive weakness and death. Medical notes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>45 yrs</td>
<td>ALS</td>
<td>Not reported. Death certificate added diagnosis (phthis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 3</td>
<td>Proband</td>
<td>62 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>54 yrs</td>
<td>ALS</td>
<td>Suspicious history reported; slurred speech and immobility over 2 years.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death certificate (Muscular dystrophy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not reported</td>
<td>Relative</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Confirmation</td>
<td>Genetics</td>
<td>FALS definition</td>
</tr>
<tr>
<td>-------------</td>
<td>----------</td>
<td>------</td>
<td>-----------</td>
<td>-------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Family 4</td>
<td>Proband</td>
<td>56 yrs</td>
<td>ALS</td>
<td>Examinned</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>50 yrs</td>
<td>ALS</td>
<td>Not reported. Death certificate added diagnosis (phthisis and asthenia x 4 months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 5</td>
<td>Proband</td>
<td>61 yrs</td>
<td>ALS</td>
<td>Examinned</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>59 yrs</td>
<td>ALS</td>
<td>Relative subsequently diagnosed. Examinned</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proband</td>
<td>58 yrs</td>
<td>ALS</td>
<td>Examinned</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td>Family 6</td>
<td>2nd degree</td>
<td>79 yrs</td>
<td>ALS</td>
<td>Not reported. Death certificate added diagnosis (dementia with immobility x 2 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>75 yrs</td>
<td>ALS</td>
<td>Not reported. Death certificate added diagnosis (respiratory failure, dementia with immobility)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 7</td>
<td>Proband</td>
<td>47 yrs</td>
<td>ALS</td>
<td>Examinned</td>
<td>Known gene</td>
<td>Definite FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>51 yrs</td>
<td>ALS</td>
<td>Suspicious history reported; slurred speech and immobility over 1 year. Death certificate (Muscular dystrophy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family</td>
<td>Relative</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Confirmation</td>
<td>Genetics</td>
<td>FALS definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------</td>
<td>-----</td>
<td>-----------</td>
<td>--------------</td>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>Proband</td>
<td>75 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>No known gene</td>
<td>Probable FALS</td>
</tr>
<tr>
<td>Family 8</td>
<td>1st degree</td>
<td>50 yrs</td>
<td>ALS</td>
<td>Not reported; death certificate (Bulbar paralysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 9</td>
<td>Proband</td>
<td>63 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>No known gene</td>
<td>Probable FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>50 yrs</td>
<td>ALS</td>
<td>Not reported; death certificate (Bulbar Palsy, MND)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 10</td>
<td>Proband</td>
<td>72 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>Not tested</td>
<td>Probable FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>38 yrs</td>
<td>ALS</td>
<td>Not reported; death certificate (Bulbar paralysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 11</td>
<td>Proband</td>
<td>74 yrs</td>
<td>ALS</td>
<td>Examined</td>
<td>No known gene</td>
<td>Probable FALS</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>68 yrs</td>
<td>ALS</td>
<td>Not reported. Death certificate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3rd degree</td>
<td>43 yrs</td>
<td>ALS</td>
<td>Suspicious history reported; slurred speech and Immobility over 1.5 years. Death certificate (Multiple Sclerosis, death from respiratory failure)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family 14</td>
<td>Family 13</td>
<td>Family 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th degree</td>
<td>6th degree</td>
<td>2nd degree</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proband</td>
<td>Proband</td>
<td>Proband</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51 yrs</td>
<td>48 yrs</td>
<td>41 yrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS</td>
<td>ALS</td>
<td>ALS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative subsequently diagnosed</td>
<td>Examined</td>
<td>Examined</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not reported; death certificate (Bulbar paralysis x 1.5 years)</td>
<td>Not reported</td>
<td>Not tested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No known gene</td>
<td>No known gene</td>
<td>Probable FALS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FALS definition:
- Probable FALS
- Suspected FALS

Confirmation:
- Examined
- Not tested

Genetics:
- Not reported

Age:
- 69 yrs
- 66 yrs
- 51 yrs

Diagnosis:
- ALS
- ALS
- ALS

Relative Age Diagnosis:
- Proband 69 yrs ALS
- Family 12 2nd degree 41 yrs ALS
- Proband 66 yrs ALS
- Family 13 6th degree 48 yrs ALS
- Proband 68 yrs ALS
- Family 14 4th degree 51 yrs ALS
There was no difference in the age at disease onset for those patients who originally reported a family history (mean age 63.5 years, SD 9.8) and those who were subsequently discovered to have a family history (mean age 64.4 years, SD 7.5; p=0.764).

6.5.3 Population based rate of FALS

In total, of 169 incident ALS patients recruited over the four-year period, 27 had a family history of ALS (16%) in any relative. When this was limited to ALS in another first-, second- or third-degree relative the rate of FALS was 14% (24/169), when limited to first- and second-degree relatives only the rate of FALS was 12% (20/169), and when limited to first-degree relatives only the rate of FALS was 6% (10/169). There is a statistical difference between the rate of ALS initially reported by patients on the register (7.7%) and the actual rate of FALS ascertained through the family history method (16.0%, p=0.018).

6.5.4 Proportion of controls reporting a family history of ALS

Six of the healthy controls (3.1%) originally reported a family member with ALS (one first-, four second-, and one third-degree relative). Three further cases of ALS were identified through death certification (three second-degree relatives). 4.7% (9/192) of healthy controls had a first-, second- or third-degree relative with ALS, 4.2% (8/192) had a first- or second-degree relative with ALS, and 0.5% had a first-degree relative with ALS.

6.5.5 Genetic testing in ALS patient participating in the study

Of the 172 patients from 169 kindreds with ALS who participated in the study, DNA was available for analysis on 155 (90%). 19 (12%) patients tested positive for a known ALS gene (17 for the repeat expansion in C9orf72, one FUS mutation, and one TARDP mutation). No patient tested positive for a known mutation in SOD1, VCP or PGN.

Neither of the patients testing positive for FUS or TARDP reported a family history of ALS, although the information available on the patient with TARDP was limited.

DNA was available for testing on 24 of the 27 cases who reported a positive family history of ALS (FALS cases). In total 45.8% (11/24) of FALS cases tested positive for the C9orf72 repeat expansion, with the remainder testing negative for any of the known ALS genes.
6.5.6 Classification methods for FALS cases

ALS patients reporting a family history of ALS were then classified as definite, probable, possible or suspected FALS as per the criteria proposed in chapter four (4.1.6). Just over half of all familial cases (14/27) had definite FALS by proposed criteria. 11 of the definite FALS cases tested positive for a known gene. This means that the three remaining families have a large number of affected members with no known gene identified. None of the probable, possible or suspected FALS cases are associated with a known gene and the majority have only two family members affected.

The frequency of affected cases within each kindred is presented in part B of figure 6.5. Just over half of FALS cases only have two affected members.

Genes known to cause ALS were identified in 41% (5/12) of families where two members are affected, and 56% (5/9) of families where three members are affected.

![Figure 6.5 A. Proportion of cases in each FALS definition category; B. Number of family members affected in each kindred.](image)

6.5.7 Relative risk of ALS

The hazard rate of ALS among first- and second-degree relatives compared to relatives of controls was stratified into three groups compared to the relatives of controls (figure 6.6):

- Relatives of ALS patients positive for the repeat expansion in C9orf72 had a HR of
34.3 of developing ALS (p<0.0001)

- Relatives of ALS patients negative for the repeat expansion had a HR of 2.3 of developing ALS (p=0.019)

- Relatives of ALS patients of unknown repeat expansion status had a HR of 6.5 of developing ALS (p<0.0001)

Of note the C9orf72 status of the patient was either known to be positive, negative or not tested. The C9orf72 status was not available on any relatives due to ethical restrictions.

<table>
<thead>
<tr>
<th>Relative of patient with unknown genetic status</th>
<th>Hazard rate</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative of C9 positive patient</td>
<td>34.3</td>
<td>18.1-64.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Relative of C9 negative patient</td>
<td>2.3</td>
<td>1.2-4.4</td>
<td>0.019</td>
</tr>
<tr>
<td>Relative of patient of unknown genetic status</td>
<td>6.5</td>
<td>2.4-18.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure 6.6 Cumulative hazard stratified into groups based on repeat expansion status demonstrated in the figure. The table gives the hazard rates for each group compared to the hazard rate for relatives of controls (HR 1.0)

6.5.8 Segregation analysis of FALS cases

Of the 27 incident patients with a family history of ALS, 24 families had a pattern of transmission of ALS consistent with dominant inheritance. The remaining cases represented
patients with ALS where a very distant relative had ALS. No anticipation, in terms of age at disease onset, was observed.

Segregation analysis of ALS transmission was carried out using the two methods discussed in section 6.3.12 (using a manual method and using an online penetrance calculator, PenCalcWeb® [215]) in all family members who lived beyond fifty years. Firstly the two methods were compared to ensure that results were similar.

Comparison of penetrance result from manual method and PenCalcWeb

![Comparison of penetrance result from manual method and PenCalcWeb](image)

Figure 6.7 Comparing penetrance estimates for each kindred using two different methods

On the whole results were comparable but the manual method did not properly account for obligate carriers who did not develop ALS over the course of their lifetime. The most marked differences in penetrance estimates can be seen for cases 6, 9, and 22. These families had 2, 3 and 3 obligate carriers respectively that were not accounted for by the manual method, and it incorrectly appears that penetrance is complete despite obligate carriage. PenCalcWeb® reports a better estimate of penetrance. This is particularly important as very few families in this cohort demonstrate complete penetrance.
The penetrance estimate for ALS from generation to generation using PenCalcWeb® was 0.44 (SD 0.24) for families with the C9orf72 repeat expansion and 0.33 (SD 0.13) in families without the C9orf72 repeat expansion, $p=0.083$.

In families with the C9orf72 repeat expansion penetrance estimates were calculated for ALS, dementia or psychiatric disease among family members within kindreds. The mean penetrance estimate for any of these conditions within families, where one member had the C9orf72 repeat expansion, was estimated to be 0.57 (SD 0.26, range 0.13-0.99).

### 6.5.9 Informative families

Through the family aggregation study, kindreds containing multiple family members with ALS but without association with a known ALS gene, were identified, suggesting a common genetic component. Further consideration is given to these candidate families for future exon sequencing work.

**Candidate 1:** Two apparently unrelated large families containing multiple members with ALS and ALS-FTD were identified (figure 6.8 A, B).
Family 1.

I

II

III

IV

MND

MND+FTD

Family 2.

I

II

III

IV

Key:

♦ Same Surname

60 MND

75 MND

80 Alzheimer’s

87

87 Alzheimer’s

55 Proband

Figure 6.8 Informative families without any known genes identified from family aggregation study (arrow denotes proband)
Family 1 (figure 6.8 A): Three individuals have ALS (II.6, IV.1, IV.5). There is an autosomal dominant pattern. Other psychiatric conditions (II.8, III.1) can be seen on the paternal side suggesting that the putative gene may cause a broad phenotype.

Family 2 (figure 6.8 B): Four affected members with ALS (III.1, III.2, III.11, IV.3), demonstrating either a recessive pattern or a dominant pattern with incomplete penetrance. Both of these families originally came from the same townland in the same county in Ireland suggesting a possible founder effect in a common ancestor. This is the same county that was identified in the epidemiology chapter (section 4.2.15), which has a rate of familial ALS that is more than two standard deviations above the national rate for familial ALS. The family in figure 6.8 B also has multiple members with primary infertility and numerous adopted members.

Candidate 2: Through the family aggregation study a large extended kindred containing multiple members with ALS (III.1, III.2, V.22, V.26) was identified. All family members originated from a small village in rural Ireland.

![Family Tree]

Figure 6.9 Family history for candidate for exome sequencing (Arrow denotes proband, ALS patients with DNA were selected for Exome sequencing)
This family first came to my attention when the proband stated that a maternal great grandfather (I.2) had been married twice and that descendants from both his first- and second-wife developed ALS. Two of his grandchildren from the first marriage developed rapidly progressive ALS (III.1, III.2). Two grandchildren (IV.1, IV.3) from his second marriage had a dementia-like illness. Three of his great-grandchildren (V.22, V.1, V.6) from the second marriage have i) upper motor neuron predominant ALS, ii) an unspecified progressive UMNL disorder of eight years duration, and iii) Parkinson's Disease. Recently a fourth member (V.26) of the kindred with ALS was identified on the paternal side of the family.

Three of the four grandparents of this proband have the same surname, and are from the same small village in rural Ireland. In fact both maternal and paternal grandfathers of the proband have the same first name and surname and were born within a decade of each other in the same small village in the nineteenth century. The pedigree for this family could represent an autosomal recessive pattern or an incompletely penetrant dominant pattern.

Initially the proband only reported one affected relative. Subsequently other family members were reported by the proband, which had previously been reported to the register as sporadic ALS cases. At the time it was noted that the family name of the proband appears very frequently on the Register leading the author to wonder if people who carried this family name might be at increased risk of carrying a mutation which caused ALS or made them more susceptible to the illness.

The surname for this family is the same one that was identified as being over represented in the ALS Register, in chapter four (section 4.2.17). The strong family history would suggest that this family may harbour a novel ALS gene.
6.6 Results of Aggregation study: Neurodegenerative and neuropsychiatric diseases

6.6.1 Analysis to look for effects of case clustering in families

The number of dementia cases in each family, for cases (n=169 families) and controls (n=192 families), was compared visually using a histogram to ensure that there was no evidence of clustering which may have driven results.

![Histogram demonstrating the frequency of dementia cases in families of cases compared to controls](image)

Figure 6.10 Histogram demonstrating the frequency of dementia cases in families of cases compared to controls

Binary logistic regression in a generalized linear model was used to compare the frequency of dementia cases in relatives of cases and controls, taking into account individual kindred size. No significant difference was found between cases and controls (p=0.127), suggesting that the effects of clustering are unlikely to affect the outcome of aggregation analysis.

Similar methods were applied to PD, schizophrenia, and suicide, and there was no statistical evidence for a cluster effect.

6.6.2 Initial analysis

Initial analysis of the rates of neurodegenerative and neuropsychiatric disorders in family members was carried out on the entire cohort; first- and second-degree relatives of 172 patients (in 169 kindreds) with ALS compared to relatives of 192 controls. The relative risk
and hazard rate (calculated by two different methods as described above) of Parkinson's disease, non-specified dementia, depression, schizophrenia, and suicide are reported in the table 6.10.

<table>
<thead>
<tr>
<th></th>
<th>Relatives of cases n=4050</th>
<th>Relatives of controls n=5634</th>
<th>Risk ratio</th>
<th>X², p-value</th>
<th>Hazard rate</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson disease</td>
<td>24 affected</td>
<td>35 affected</td>
<td>1.0</td>
<td>0.3, p=0.858</td>
<td>0.8</td>
<td>p=0.298</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td>Dementia</td>
<td>152 affected</td>
<td>186 affected</td>
<td>1.1</td>
<td>1.4, p=0.255</td>
<td>1.2</td>
<td>p=0.052</td>
<td>0.9-1.4</td>
</tr>
<tr>
<td>Depression</td>
<td>24 affected</td>
<td>31 affected</td>
<td>1.1</td>
<td>0.1, p=0.891</td>
<td>1.1</td>
<td>p=0.684</td>
<td>0.7-1.7</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>9 affected</td>
<td>3 affected</td>
<td>4.2</td>
<td>5.4, p=0.041</td>
<td>4.7</td>
<td>p=0.004</td>
<td>1.6-12.3</td>
</tr>
<tr>
<td>Suicide</td>
<td>14 affected</td>
<td>4 affected</td>
<td>4.9</td>
<td>9.6, p=0.004</td>
<td>5.6</td>
<td>p=0.000</td>
<td>2.4-12.9</td>
</tr>
</tbody>
</table>

Table 6.10 Comparison of relatives, of all cases and all controls, demonstrates a significant relative risk of schizophrenia and suicide in relatives of all ALS patients compared to relatives of controls

6.6.3 Subgroup analysis; stratifying for the C9orf72 repeat expansion

The cohort included 138 C9orf72 repeat expansion negative patients, 17 C9orf72 repeat expansion positive patients, and 17 patients with unknown gene status. Subgroup analysis was carried out using Cox-proportional hazard analysis to compare disease risk between the following sub-groups:

- 3,335 relatives of 138 C9orf72 repeat expansion negative patients, and
- 401 relatives of 17 C9orf72 repeat expansion positive patients

Compared with:

- 5,634 relatives of 192 controls

The results are displayed in table 6.11. The hazard rate in all diseases in controls is set at 1.0.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Group</th>
<th>Hazard rate</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson's disease</td>
<td>Relative of C9 positive patients</td>
<td>1.3</td>
<td>0.5-3.7</td>
<td>0.570</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9 negative patients</td>
<td>0.7</td>
<td>0.4-1.1</td>
<td>0.126</td>
</tr>
<tr>
<td>Dementia</td>
<td>Relative of C9 positive patients</td>
<td>1.6</td>
<td>1.1-2.4</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9 negative patients</td>
<td>1.2</td>
<td>0.9-1.4</td>
<td>0.100</td>
</tr>
<tr>
<td>Depression</td>
<td>Relative of C9 positive patients</td>
<td>3.3</td>
<td>1.6-7.0</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9 negative patients</td>
<td>0.6</td>
<td>0.3-1.1</td>
<td>0.075</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>Relative of C9 positive patients</td>
<td>18.2</td>
<td>4.7-71.2</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9 negative patients</td>
<td>4.7</td>
<td>1.6-14.0</td>
<td>0.006</td>
</tr>
<tr>
<td>Suicide</td>
<td>Relative of C9 positive patients</td>
<td>16.6</td>
<td>5.6-49.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Relatives of C9 negative patients</td>
<td>5.1</td>
<td>2.2-12.1</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 6.11 Comparison of relatives of (A) C9 positive cases with controls, and (B) C9 negative cases compared with controls in a Cox-regression proportional model

There was no difference in the rate of Parkinson's disease (PD) between the three groups. The hazard rate for dementia and depression were increased for relatives of ALS patients carrying the repeat expansion compared with relatives of those not carrying the repeat expansion and with relatives of controls. The hazard rate of all relatives of patients was increased for schizophrenia and suicide compared with controls and the hazard rates were higher for relatives of cases carrying the repeat expansion than relatives of cases not carrying the repeat expansion.

Direct comparisons, using the Cox proportional hazard method, were made between the rate of schizophrenia and suicide in relatives of patients with and without the repeat expansion. The hazard rate for suicide was increased in relatives of patients with the repeat expansion compared to relatives of patients without the repeat expansion (HR 3.4, 95% CI 2-5.6;
p<0.0001), but the hazard rate did not reach statistical significance for the rate of schizophrenia between the two groups (HR 2.1, 95% CI 0.96-4.8; p=0.06).

[Of note, comparisons were also made between relatives of familial ALS cases with the repeat expansion and those without the repeat expansion, and the rate of dementia was not increased in the non-C9orf72 FALS cases (RR 1.2 p=0.423).]

6.6.4 Subgroup analysis; Cognitive testing

Of the 172 cases, 102 had undergone cognitive testing as part of the longitudinal study of cognition. Patients carrying a C9orf72 repeat expansion or who had not been tested for known genes were excluded from this analysis. 83 ALS patients, negative for the C9orf72 repeat expansion had undergone cognitive testing. 37 patients had normal cognition, and 46 patients had evidence of abnormal cognition (more than two standard deviations below the mean on two or more tests).

Analysis was carried out to compare the rate of dementia recorded in 975 relatives of the 37 cognitively normal patients, and the 837 relatives of the 46 ALS patients with abnormal cognition. The relatives of the cognitively impaired ALS patients without a C9orf72 repeat expansion had an increased hazard rate of dementia of 2.6 (95% CI 1.5-4.2; p=0.001), compared to the relatives of the cognitively normal ALS patients without a C9orf72 repeat.

The relatives of non-C9 ALS patients with cognitive impairment also had an increased hazard ratio for developing ALS compared to relatives of non-C9 ALS patients without cognitive impairment and controls. A hazard ratio for developing ALS was 4.4 (95% CI 2.7-7.3, p<0.0001) in relatives of non-C9 ALS patients with cognitive impairment, and 1.6 (95% CI 0.8-3.0, p=0.18) in relatives of non-C9 ALS patients with normal cognition, compared to controls.
Figure 6.11 Hazard ratio of developing ALS in relatives of ALS patients with normal and abnormal cognition compared to controls

6.6.5 Subgroup analysis; age at onset of disease

Age of disease onset in relatives was recorded for all cases of PD and dementia where information was available. There were no significant differences in the age of onset of PD between the groups. Relatives of C9orf72 positive patients had an increased rate of dementia compared to controls (HR 1.6, p=0.017). The age of onset of dementia in the relatives of C9orf72 positive cases was 70.1 years (SD 3.0 years) compared to 80.3 years (SD 0.9 years) in relatives of controls, p<0.0001 (see figure 6.12).

Relatives of ALS cases without the C9orf72 repeat expansion have a marginally increased relative risk of dementia compared to relatives of controls (HR 1.2), although this did not reach statistical significance (p=0.100). There was a significant difference in the age of onset of dementia in the relatives of cases without the C9orf72 repeat expansion of 74.6 years (SD 1.3 years) compared with 80.3 years (SD 0.9 years) in relatives of controls (p=0.003).
A. Onset of Dementia in relatives of C9orf72 positive patients compared to relatives of controls

B. Onset of Dementia in relatives of patients not carrying C9orf72 compared to relatives of controls

Figure 6.12 Age at onset of dementia in relatives of patients is younger compared to relatives of controls

There were no data available regarding the age at onset of schizophrenia in cases or controls. The age at which relatives of cases and controls died from suicide was the same in each group (median age 41.0 years in relatives of cases and 41.5 years in relatives of controls, p=0.958).

6.7 Results of Aggregation study: Fecundity

The number of offspring between cases and controls was compared. Comparisons were made between cases and controls that had ever been married, taking marriage as a proxy for the wish to produce offspring (analysis for the whole cohort, ever married and never married, yielded the same results). The same proportion of cases and controls were married; 88.4% of the cases (152/172) were married compared with 89.5% of the controls (172/192, p=0.74). The median number of offspring differed between cases and controls (2 and 3 respectively) but this did not reach statistical significance (p=0.67).

Analysis was performed to look for differences in the distribution of frequency of offspring between cases and controls. Figure 6.13 (part A) shows the actual frequency of children for
cases and controls. Analysis of the frequency distribution of offspring between the two
groups does not reach statistical significance ($X^2=24.2, p=0.1$).

A. Table

<table>
<thead>
<tr>
<th>Number of children</th>
<th>Cases (n=152)</th>
<th>Controls (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>Percent</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>16.2</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>10.5</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>23.7</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>19.2</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>15.1</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>7.9</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>4.6</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>172</td>
</tr>
</tbody>
</table>

B. % Frequency of offspring in cases

C. % Frequency of offspring in controls

Figure 6.13 A. Table demonstrating the frequency of offspring from cases and controls. B, C
Histograms depicting the percentage frequency of offspring between cases and controls

Figure 6.14 shows the proportional frequency of offspring in cases versus controls side by
side on the same bar chart. The proportional frequencies illustrated here strongly suggest
that cases are more likely than controls to have one or fewer children. The frequency of one
child or fewer was compared in cases versus controls; 23.6% (36/152) of cases had one child
or fewer compared to 12.8% (22/172) of controls ($p=0.01$). There were no differences when
comparisons were made for gender of the participant.
In order to assess for the impact of carrying a $C9orf72$ repeat expansion, comparisons were made between frequency of offspring in controls and $C9orf72$ repeat expansion carriers, and also between controls and non-$C9orf72$ repeat expansion carriers.

There was no statistical difference in the overall frequency distribution between controls and non-$C9orf72$ repeat expansion carriers ($p=0.23$), but there was a difference between the frequency of one or fewer children in controls (22/172, 12.8%) versus non-$C9orf72$ repeat expansion carriers (27/123, 22%; $p=0.037$). No differences were found between numbers of offspring for males and females in this group.

A comparison was made between the number of offspring between married cases carrying the $C9orf72$ repeat expansion (14) and controls (172). The difference in the frequency distribution was not significant ($p=0.23$) but when the cohort was split by gender there was a significant difference in the frequency distribution of offspring between female $C9orf72$ repeat expansion carriers and female controls ($p=0.028$). This difference was primarily explained by the high proportion of women carrying the $C9orf72$ repeat expansion with one child or fewer. 62.5% (5/8) of women carrying the repeat expansion had one or fewer children compared to 9.9% (8/81) of female controls ($p<0.0001$).
1.7% (3/172) of controls adopted children compared with 4.0% (6/152) of cases. This finding was not significant (p=0.3).

6.8 Discussion

6.8.1 General points

This is a large family aggregation study of 364 ALS cases and controls, designed to establish the rate of ALS, PD, dementia, schizophrenia, and suicide in 9,684 first- and second-degree relatives.

This cohort comprises an unbiased sample of incident cases of ALS from a population-based register over a four-year study period, which is well matched with the control group. Patients with ALS who participated and completed the questionnaire were younger and survived longer than those who agreed to participate and were unable to complete the questionnaire. This bias has been demonstrated in other population-based studies[7], and is unavoidable given the rapid progression to death in some patients with ALS.

Data collection from controls yielded higher rates of complete data collection and lower rates of incomplete data collection compared to the questionnaires of cases. Disability and fatigue among patients and their caregivers was the main reason cited for recording histories with fewer relatives.

6.8.2 Verification method

This study verified the cause of death with official death certificates in 2,494 relatives. No other study to date has attempted to verify cause of death with certification. Self-reporting by patients was accurate overall, with a positive predictive value of 80% when comparing all reported causes of death to official death certificate. However the positive predictive value of reported neurological cause of death reported by family history method compared to official certified cause of death was only 20%. A true comparative between the family history method and death certificates cannot be made, as neither are 100% accurate. In the last
century in rural Ireland, a county clerk often completed the official death certificate. Many years ago elderly people did not seek medical attention when in decline, and were often reported to die of "old age". When clarifying cause of death with family members of relatives who died many decades ago, a very detailed description of a dementing process may have been given that was not recorded on a death certificate. It is hard to overlook a good clinical description, despite the death certificate reporting the cause of death as "heart failure". This is a problem inherent to taking family histories on previous generations.

Obtaining death certificates did add power to the study as they added cause of death in 830 cases where the cause of death was unknown by the relative, including 165 previously unreported neurological causes of death.

In a number of pedigrees in which a dominant pattern of inheritance was observed and an ALS gene was known to exist (C9orf72), patients described symptoms consistent with ALS in relatives, but a non-neurological cause of death was given in the certificate or another neurological cause was reported. One C9orf72 positive ALS kindred with autosomal dominant inheritance through the paternal line had two relatives with symptoms consistent with ALS. One first degree relative died at the age of 48 years with a death certificate that stated 'Secondary Polio' as the cause of death. Review of the medical notes demonstrated that although this person had suffered from Polio in childhood, that they died from a progressive upper and lower motor neuron condition resulting in respiratory failure and death which was consistent with a diagnosis of ALS. A second relative was reported as dying at the age of 45 years from a wasting disease, which had caused a deterioration in walking and speech over the course of two years. The cause of death reported on the death certificate was 'phthisis' and was reported in the 1940s (phthisis is defined as a disease characterised by the wasting away or atrophy of the body or part of the body or tuberculosis of the lung).

Another C9orf72 positive ALS patient reported that a parent had had died from multiple sclerosis in their fifties. Upon further questioning, it transpired that he had been in good health all of his life until he had developed problems with speech and swallow two years prior to his death in the nineteen seventies. A couple of months after developing problems...
with speech and swallow he became bedbound, and died shortly afterwards from pneumonia. Based on the history and genetic results this almost certainly represents a case of ALS, but the proband reported Multiple Sclerosis and death certificate reported 'Muscular dystrophy' as the cause of death.

Examples of other causes given where the history and genetic results suggest ALS are as follows:

'Dementia with immobility for two years'
'Spinal paralysis for two years'
'Muscular dystrophy'
'Secondary polio'
'Progressive muscular atrophy (of duration 15 months)'
'Asthenia and phthisis'
'Atrophy and exhaustion'
'Bulbar paralysis'

In a number of cases where the death certificate reports ALS or MND as the cause of death, patients and controls report a different cause of death. In one large FALS pedigree, where multiple affected members carried the C9orf72 repeat expansion, it was reported that a relative from a previous generation had suffered from 'crippling arthritis and rheumatism', when in fact the death certificate said MND. A second relative was said to have suffered from Multiple Sclerosis but the death certificate said MND (with upper motor neuron predominance).

In the case of four ALS patients who did not originally report a family history of ALS, but who were unaware of the cause of death in other relatives of previous generations, death certification revealed the cause of death as being 'bulbar paralysis'. None of the death certificates from relatives of controls reported bulbar paralysis as a cause of death (0/1560 compared to 4/934, p=0.0097).

It is evident from this study that neither verbal family histories nor death certificates are entirely accurate. It is vital that every effort is made to get death certificates in a family
aggregation study, especially if there is uncertainty about a diagnosis or in cases where no information is available.

### 6.8.3 Familial ALS

This is the first study to estimate the true rate of familial ALS in a prospective incident based cohort. The meta-analysis in chapter three (section 3.2), estimated that the reported rate of FALS on a number of prospective population-based registries was 5.1%. This study used a family history questionnaire with follow-up contact, genealogical searches, and death certification to estimate the true rate of familial ALS: the rate of familial ALS in this cohort is 16% when all relatives (any reported degree of relative) are included, 14% when first-, second- and third-degree relatives are included, 12% when first- and second-degree relatives are included, and 6% when first-degree relatives only are included.

The frequency of affected members in families was the same as reported by Valdmanis [51], with over half of all ‘familial’ kindreds only having two affected members. The criteria for familial ALS proposed in chapter four were used to separate familial cases into definite, probable, possible and suspected familial ALS. There were 14 cases of definite familial ALS, of which 11 had a known gene. The remaining three families did not carry a known gene but had a large number of closely related family members affected with ALS. The classification system allows rapid identification of familial cases without known genes, which may be useful in future genetic studies.

The proportion of controls predicted to have a family history of ALS by chance (~4%) is almost identical to the observed rate (4.1-4.7%) of ALS among relatives of controls in this study. This confirms the theoretical prediction (section 3.1.6) that a proportion of familial ALS cases occurring within the same kindred may be due to chance co-occurrence rather than to a genetic or epigenetic cause. Patients with probable FALS (one or more third degree relative affected), and suspected FALS (one or more forth degree relatives) account for ~3.5% (of 16%) of the FALS cases. It is most likely that a high proportion of these cases are due to chance co-occurrence rather than another cause, with the remainder of the cases occurring by chance in the probable category (one first or second degree relative affected).
Chance co-occurrence may explain the non-segregation of SOD1 genes previously reported in kindreds[123]. This finding is important as geneticists searching for new ALS genes may include FALS cases where only two people in the family are affected. The genetic yield from these families is likely to be lower as chance co-occurrence is higher. The proposed criteria for the definition of FALS aim for consistency between studies regarding the definition of definite and probable FALS, which are the groups likely to yield positive results in genetic studies for Mendelian genes of major effect.

The lifetime risk for ALS in the general population is estimated to be 1 in 350 in men and 1 in 472 in women (average 1/411) [95]. This study estimates a hazard rate for ALS of 34 among first- and second-degree relatives of patients with the repeat expansion compared with relatives of controls. The hazard rate for ALS among relatives of patients with no repeat expansion is only 2.3, which means that their lifetime risk is 1 in 200 compared with 1 in 400 in the general population. This is the first time that this stratification has been reported for ALS risk, and is an important distinction to make for family members coming to the neurologist's office with worries about their own personal risk.

The penetrance estimate for ALS from generation to generation using PenCalcWeb® in relatives who lived to age fifty and above was marginally higher (0.44) in ALS cases where the causative gene was the repeat expansion in C9orf72. The difference in results obtained using the manual method and the online penetrance calculator were reported, particularly the failure of the manual method to account for lifelong obligate carriage which resulted in a falsely elevated penetrance value in a number of cases. This may explain, in part why the reported penetrance for C9orf72 varies between studies.

In families with the C9orf72 repeat expansion penetrance estimates were calculated for ALS, dementia or psychiatric disease among family members within kindreds. The mean penetrance estimate for any of these conditions within families, where one member had the C9orf72 repeat expansion, was estimated to be 0.57 (SD 0.26, range 0.13-0.99).

There are limitations to findings in this study. The rate reported is population specific, as the study drew from an Irish population-based cohort of ALS patients and matched Irish controls.
Genetic factors such as local founder effect and genetic homogeneity within a population may influence the observed rate of FALS. Secondly, in a number of cases the cause of death reported on the death certificate was used. In the past, many cases may have been missed due to poor access to medical services and to lack of recognition of the disorder. ALS was previously called creeping paralysis (limb onset), and bulbar paralysis (bulbar onset). Prior to the availability of medical imaging it may have been difficult to determine the difference between multiple sclerosis and atypical ALS. This study included a number of cases where male relatives who died fifty or more years ago were described as having a rapidly progressive muscular problem with associated dysarthria but without bladder or bowel dysfunction, with onset between the ages of 50 and 70 years, and who died of respiratory failure within one to two years of onset, whose illness was described as “multiple sclerosis” or “muscular dystrophy” on death certificates.

6.8.4 Aggregation of Neurodegenerative and Neuropsychiatric diseases

This study compares the presence or absence of neurodegenerative and neuropsychiatric conditions in 4,050 first- and second-degree relatives of 172 population-based incident ALS cases and 5,634 first- and second-degree relatives of 192 age- and sex-matched controls. Relatives of ALS cases have a hazard rate of dementia of 1.2 (p=0.052) compared to relatives of controls. The relative risk method results in a similar value, but does not reach significance (RR 1.3, p=0.255).

When stratified subgroup analysis is carried out on relatives of ALS cases with a C9orf72 repeat expansion, the result is an increased hazard rate of dementia of 1.6 (p=0.017). This is not an unexpected finding as the presence of the C9orf72 repeat expansion is associated with development of either ALS, FTD or the combination of ALS-FTD in affected individuals. The age of onset of dementia is ten years younger in relatives of patients with the C9orf72 repeat expansion. These findings reflect the high rate of FTD seen among relatives of ALS patients with a C9orf72 repeat expansion. It remains to be seen which genetic, epigenetic and environmental factors will be associated with the differentiation into one condition or the other.
In section 6.6.4, subgroup analysis comparing relatives of non-C9 patients to relatives of controls, demonstrates that there is evidence for another unknown gene associated with both dementia and ALS. When a distinction is made between relatives of cognitively impaired non-C9 ALS patients, and relatives of cognitively normal non-C9 ALS patients, the hazard ratio of dementia among relatives is elevated among relatives of ALS patients with cognitive impairment (HR 2.6). The hazard ratio for developing ALS among relatives of cognitively impaired ALS non-C9 patients is also much higher compared to relatives of cognitively normal non-C9 ALS patients and relatives of controls (HR 4.4). Genetic studies should focus on non-C9 families where the proband is cognitively impaired, and there is a family history of ALS or dementia. This highlights the benefits of using an aggregation study to identify new subgroups to utilize in genetic studies.

Despite previous case reports of ALS and schizophrenia co-occurring in families, this is the first family aggregation study to report an increased rate of schizophrenia among relatives of patients with ALS. The rate of schizophrenia is increased in relatives of C9 and non-C9 ALS patients.

A previous family aggregation study of conditions occurring among relative of patients with FTD demonstrated an increased morbid risk for schizophrenia in relatives of patients with FTD compared to relatives of patients with Alzheimer's disease[172]. Those findings are similar to the findings in this study: the authors reported a relative risk of schizophrenia of 4.1 among 741 first-degree relatives of one hundred FTD patients (the relative risk of schizophrenia in relatives of all ALS patient in the family aggregation study in this study is 4.2). While FTD and schizophrenia are both neurobehavioural syndromes characterised by an alteration in social interaction with varying degrees of frontal dysfunction, the two syndromes differ in that the age of onset is markedly younger in schizophrenia, the course is more variable, and hallucinations and delusion are prominent features, compared with an older age of onset with progressive decline in FTD, and delusions reported much less commonly. Because of similarities between FTD and schizophrenia, Schoder and colleagues[172] examined each relative diagnosed with schizophrenia, wherever possible,
and confirmed that these relatives did indeed have schizophrenia rather than a misdiagnosed case of FTD. They also reported three families where schizophrenia and FTD segregated with PRGN and VCP mutations, both of which are also associated with ALS. None of the ALS patients in our study tested positive for either of these genes. The study by Schoder and colleagues was carried out before the discovery of the C9orf72 repeat expansion, which is associated with one-third of cases of FTD, and so was unable to discern whether relatives of FTD patients with the C9orf72 repeat expansion had a higher rate of schizophrenia.

Lillo and colleagues[165] recently reported that patients with ALS-FTD were more likely to report delusions than patients with FTD alone (increased rate of delusions was 50%; odds ratio, 4.4), suggesting the possibility that the underlying pathological mechanisms that result in the development of delusions may overlap between schizophrenia and ALS-FTD.

The overlap independently identified between ALS and FTD[127], FTD and Schizophrenia[172], and ALS and Schizophrenia is compelling evidence for a link between these conditions. The reasons for are likely to be varied: there may be a specific gene resulting in a wide and varied phenotype; there may be common pathological mechanisms that result in different phenotypes depending on the interaction between other genes and environment; or these diseases may simply result from abnormal protein aggregates mediated by a similar mechanism but depositing in regions that specifically cause anterior horn cell dysfunction, frontal lobe dysfunction, and altered synaptic connectivity.

All of these conditions are brain disorders, some of which have predominantly physical manifestations, and others predominantly psychiatric manifestations, but ultimately caused by some process resulting in neuronal dysfunction.

Family members of patients with ALS have a higher rate of schizophrenia and suicide compared to relatives of controls. Choosing ALS patients with a family history of schizophrenia might yield a susceptibility gene for both conditions. Before genetic analysis is undertaken, further work is required to look at ALS probands with a family history of schizophrenia, and to determine whether any psychiatric phenomena are seen in the ALS probands, and to determine whether abnormalities are found on physical examination and on
electrophysiological studies of the relatives with schizophrenia. From this study it is not possible to determine the how definitive was the diagnosis of schizophrenia in the affected relatives. Records were unavailable, and it is possible that these patients were in fact young people presenting with an early onset frontotemporal syndrome, which can be misdiagnosed as schizophrenia. Schoder and colleagues, by interviewing the relatives diagnosed with schizophrenia, were able to confirm that this they were not merely misdiagnosed cases of early onset FTD. In our family aggregation study the histories reported strongly suggest schizophrenia rather than early onset FTD. A disproportionate number of ALS patients in this study reported that they had relatives who “disappeared in their twenties/thirties”, or who were “always odd”, or spent many years in a psychiatric hospital. None of these were classified as a particular condition as the diagnosis was unknown, however from this aggregation study there is definitely evidence for aggregation of psychiatric conditions and ALS. These personality traits may represent a ‘behavioural endophenotype’ that could be used in the future in genetic studies.

The rate of suicide was markedly increased among relatives of ALS patients, again irrespective of \textit{C9orf72} repeat expansion status, however the relative risk of depression was not elevated in relatives of patients with no \textit{C9orf72} repeat expansion compared to relatives of patients with a \textit{C9orf72} repeat expansion. In Schoder and colleagues' study reporting an increased rate of schizophrenia among relatives of patients with FTD they report that 70% of all schizophrenic patients in their cohort had attempted suicide on at least one occasion, with 15% completing suicide. The timing of suicide in relatives of ALS patients in our study was independent of the timing of the diagnosis of ALS and in nearly all cases preceded the onset of ALS in another relative. Suicide is often associated with depression, but may independently be associated with impulsivity, a highly heritable neurobehavioural trait[217]. The higher rate of suicide among relatives of patients with the \textit{C9orf72} repeat expansion may reflect increased impulsivity among patients who have developed, or will go on to develop FTD, or it may be a complex behavioural endophenotype occurring in the family. Suicide was first noted to be heritable many years ago. In a study 4% of people who completed suicide had a first
degree relative who had also completed suicide[218]. Impulsivity is something that could be addressed in more detail among family members of ALS patients. Addiction to alcohol is correlated with suicide. This family aggregation study did not specifically look at alcohol addiction among family members.

The finding that schizophrenia and ALS aggregate in families may indicate that they share a common underlying mechanism, and raises the question of whether antipsychotic drugs may be beneficial in the treatment of ALS?

An opinion piece by Stommel and colleagues[219] proposes that ALS is seen less frequently than expected in patients with schizophrenia, and that this is because antipsychotic medications may protect neurons from inflammation and stop the development of ALS. Stommel also reports the results of a Polish study[220], which demonstrated that the rate of clinical progression was reduced in patients with ALS taking pimozide (an antipsychotic and calcium channel blocker) compared to the rate in ALS patients taking selegiline and vitamin E. This is the only literature available on a topic that needs further consideration.

There are several barriers to the accurate determination of the rate of neurodegenerative and psychiatric disorders among relatives of patients with ALS compared to relatives of controls. The rate of general 'dementia' was compared between relatives of patients and relatives of controls. The scope of this project did not allow us to distinguish between FTD, primarily amnestic syndromes, or vascular dementia in relatives. In the absence of these distinctions we were still able to report that relatives of patient with ALS develop dementia at a younger age than relatives of controls.

Whether or not the person completing the questionnaire reported a family history of psychiatric illness obviously depended on whether they were aware of the diagnosis. This was based on a semi-structured interview, however no specific diagnostic tool was used. A recent paper by Hardt and Franke[221] demonstrates high reliability and validity of relatives' reports of major psychiatric disorders such as schizophrenia and addiction. In this study, a number of cases of psychotic disorders among relatives were reported by the proband, but a diagnosis of schizophrenia could not be confirmed. In these cases a diagnosis of 'unspecified
psychiatric disease with psychosis' was recorded. Unconfirmed rates of psychotic disorder were also higher among relatives of cases than among relatives of controls.

This study is first to demonstrate the increased rate of neuropsychiatric conditions among relatives of patients with ALS. Further work on this link between the pathophysiology of ALS and neuropsychiatric disease may lead to new discoveries in the fields of protein aggregation and neurogenetics.

6.8.5 Aggregation of other diseases

The rate of cardiac death is exactly the same in both groups in this study (26.6% compared to 26.9%), although the rate of death due to stroke is marginally lower in the relatives of cases (7.1%) compared to relatives of controls (8.4%).

When compared to the Central Statistics Office of Ireland, vital statistics for 2011 (www.cso.ie/en/media/csoie/releasespublications/documents/vitalstats/2011/vstats_q42011.pdf [222]), the rate of MI is similar (16.3% died from MI in CSO figures, 15.8% of relatives of ALS patients died from MI, and 15.9% of relatives of controls died from MI), as is the rate of stroke (7% died from stroke in CSO figures, 7.1% of relatives of ALS patients died from stroke, and 8.4% of relatives of controls died from stroke).

This differs from a recent study Huisman and colleagues [112], which reports a lower rate of cardiovascular diseases (stroke and MI), in relatives of cases compared to relatives of controls (relative risk 0.73, 95% CI 0.57-0.94). The family aggregation paper by Huisman and colleagues, which demonstrated the decreased rate of cardiovascular disease among family members of patients with ALS, was published in 2011, two years after this family aggregation study was designed and initiated. Therefore the questionnaire used in my family aggregation study and subsequent patient/control contact did not specifically ask about cardiovascular disease in living family members.

The death rate due to cancer is also higher among relatives of ALS patients compared to controls (21.3%, compared to 18.3%). This increase in incidence has never been reported before, although as it was not a specific aim of this study. Only one report in the literature raises the question of a link between ALS and cancer. In a report by Tan and colleagues[223],
they comment on the association of FUS with both solid cancer, and ALS. They examined gene expression in relation to FUS and demonstrate that a number of genes associated with neurological conditions, including MECP2 which causes Rett syndrome in girls, and TARDBP, are activated by FUS. Although FUS was originally associated with Ewing sarcoma[124], more recently it has been implicated in the up regulation of androgen receptors in prostate cancer[224]. If a mutation in FUS or another ALS-associated gene were to regulate a common pathway associated with both cancer and ALS, then the observation that the rate of cancer is higher among relatives of ALS patients than among relatives of controls may be valid.

6.8.6 Selection of candidates for exome sequencing project

Two different families with multiple affected family members have been described which are suspected to harbour novel genes. Below is a discussion of whether these families may be informative for exome sequencing.

Candidate 1: Two families with multiple affected family members were discussed. Family 1 from figure 6.8 has three affected members, two siblings (IV.1, IV.5) and one parent (II.6), in an autosomal dominant pattern. DNA samples are available on the two affected siblings (IV.1, IV.5) and other non-affected family members. As the two siblings share 50% of their DNA refining the discovered variant set down to a manageable number by assessing overlap between siblings will be difficult. Therefore these cases have not been chosen for exome sequencing.

Family 2 in figure 6.8 B has four affected members (III.1, III.2, III.11, IV.3). DNA samples are available on two of the members (III.1, IV.3). These two members are fourth degree relatives and only share 6.3% of their DNA. As they are distant relatives, they are good candidates for exon sequencing. Samples from this family have been sent for exome sequencing, however this technique will not capture a repeat disorder in an intronic region.

Candidate 2: In the results section family 3 was described, where a grandfather (I.2) had married twice, and had multiple offspring from both unions with ALS (III.1, III.2, V.22, V.26). DNA samples are available on two affected members (III.1, V.22) from this large kindred and
multiple unaffected individuals. The affected members are 5th degree relative which means that they share only 3.1% of their DNA. The inheritance pattern for this kindred (figure 6.9) could either be recessive or dominant. DNA from these two fifth degree relatives has been selected for exome sequencing. Other affected and unaffected members are also willing to partake which will allow thorough validation of any putative gene discovered.

6.8.7 Genetic counselling in FALS

On the basis of this study it is known that almost half of all reported familial cases of ALS (44%) are associated with a hexanucleotide repeat expansion on chromosome nine. While it is possible to discuss risk with family members of affected individuals, offering a genetic test was unlikely to add any further information. Upon learning about the discovery of the C9orf72 repeat expansion some family members were anxious to be tested for the gene to find out if family members are at risk. Genetic counselors have adopted the Huntington's disease (HD) procedure for predictive testing, which involves two pretest sessions with the person followed by gene testing, result delivery and a post result session (personal correspondence).

Testing for the C9orf72 repeat expansion differs from HD predictive testing in that a repeat expansion in HD is associated with a 100% risk of developing HD. Based on the results of this study the penetrance of ALS with a C9orf72 repeat expansion is estimated at 0.44, while the penetrance of ALS, FTD or psychiatric disorder is estimated at 0.57. Even if an asymptomatic person was to test positive for the C9orf72 repeat expansion it is impossible to determine which phenotype, if any, would be expressed (ALS, ALS-FTD, FTD, psychiatric variant).

It is important to explain to unaffected relatives who request genetic counselling that even if they are offered testing and test positive for an expanded repeat that there is no way of predicting whether they will or will not get any of a number of conditions associated with the gene. Conversely, if a patient tests negative for the repeat expansion, relatives may still be at risk from other, unknown genes, if penetrance in that family is high. There is also evidence to suggest that some patients with a repeat expansion carry a second pathological gene (the 'oligogenic' theory).
At present there are no therapeutic trials in progress for asymptomatic, gene positive relatives and so predictive testing in these individuals may be of limited benefit. If a person does decide to go forward with testing it is important that their expectations are discussed and that they are aware that a negative result will not be 100% reassuring.

In the case that the relative does not wish testing but the gene status of their relative is known, the results of this study show that risk of developing ALS is only marginally higher in relatives of ALS patients without the repeat expansion.

6.8.8 Fecundity

To date only one study has reported a reduction in offspring among male ALS patients with a family history of ALS[225]. However no studies have reported a decrease in offspring number among sporadic ALS patients. The impaired ability to reproduce has been reported in other neurological diseases associated with nucleotide repeat expansions. The mechanism of limited fertility in neurological repeat expansion disorders remains unclear, however fertility in pre-mutation carriers (55-200 repeats) of the fragile-X gene is impaired due to premature ovarian failure and altered reproductive function may also precede ovarian failure by many years thus limiting offspring number even further[150]. Recently altered reproductive function has been described in females with myotonic dystrophy[149]. 23.6% of married patients with ALS have one or fewer offspring compared to 12.8% of married controls. Given that there is no between-group difference in the number of higher order births, it is probable that a subgroup of ALS patients have fertility problems. This difference is partly accounted for by the subgroup of female ALS patients who carry the $C9orf72$ repeat expansion but this does not entirely account for the difference as the disparity is still present when the $C9orf72$ cohort are removed from analysis (22% of non-$C9orf72$ patients have one or fewer children compared to 12.8% controls).

Almost two-thirds of female ALS patients with a $C9orf72$ repeat expansion had one or fewer children and, based on the evidence of other repeat expansion disorders it is possible that limited fecundity in this group may be due to a similar mechanism. Further study of the rates
of premature ovarian failure in ALS patients with a \textit{C9orf72} repeat expansion is necessary to further examine this theory.

Patients without a repeat expansion have a higher frequency of one or fewer children than controls. ALS is a disease of middle age. Fewer than 10\% of patients develop the disease before the age of 45. It is unlikely that reproduction in ALS patients is limited by the development of motor or cognitive symptoms before they have the opportunity to reproduce. It is also unlikely that ALS patients consciously limit family size due to perceived risk to offspring.

There are limitations to these observations. Marriage is used as a proxy for the intent to reproduce. This method has been used in other studies reporting fecundity rates. Analysis was also carried out on all cases and controls, including those who had never married, and the results were the same. The cut-off point of one or fewer patients is an arbitrary figure informed by visual inspection of the histogram. The number of cases in this incident based cohort is relatively small since only 9\% of ALS patients carry the \textit{C9orf72} repeat expansion.

One could consider ALS patients with limited fecundity for further genetic studies however these samples would not be suitable for exome sequencing as the genetic abnormality would most likely be a large repeat expansion and these mutation types are not detected by exome sequencing.

\textbf{6.9 Conclusion}

This is a large family aggregation study looking at family history among incident patients with ALS compared to controls. The estimated true rate of FALS is based on multiple methods of verification, and is higher than previously reported. Despite this finding, the hazard rate for ALS among relatives of ALS patients without the \textit{C9orf72} repeat expansion is only twice the risk of relatives of controls, and in terms of absolute numbers this represents a small rise in lifetime risk.

Death certification allowed us to increase the power of the study by including relatives who died of causes of which the participant was unaware, and also assisted in verifying reported causes of death. This study segregates for the presence or absence of the \textit{C9orf72} repeat
expansion and demonstrates that the rate of dementia is higher among relatives of ALS patients with a repeat expansion. This study demonstrates a substantial burden of psychiatric illness including depression, schizophrenia and suicide in relatives of ALS patients with the \textit{C9orf72} repeat expansion. The \textit{C9orf72} repeat expansion is already known to have a variable phenotype and can cause ALS, FTD or ALS-FTD. Results from this paper suggest that neuropsychiatric conditions may also segregate with the repeat expansion.

<table>
<thead>
<tr>
<th>Accepted rate of FALS</th>
<th>Reported rate of FALS (meta-analysis)</th>
<th>Rate of FALS predicted to occur by chance</th>
<th>Rate of FALS in family aggregation study</th>
<th>Rate of family history of ALS in healthy controls</th>
<th>True rate of FALS (actual rate minus background rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>5.1%</td>
<td>4.1%</td>
<td>16%</td>
<td>4.1%</td>
<td>12%</td>
</tr>
</tbody>
</table>
Chapter 7  Summary, discussion and further work

7.1 Summary of findings

7.1.1 Familial ALS and family aggregation study

Prior to the research comprising this thesis, the definition for the term 'familial ALS' was unclear, and the rate of FALS was presumed to be 10%. The studies outlined herein have challenged this assumption, and through the use of questionnaire methodology, systematic literature review, and meta-analysis, have sought to consolidate and clarify the current state of knowledge of familial ALS before setting out to estimate the true rate through the method of a family aggregation study.

It is clear that there is no consensus for the definition of familial ALS. In particular there was a disparity between the definition most commonly used by North American investigators (familial ALS occurs when another first-degree relative is affected), and European investigators (familial ALS occurs when another first- or second-degree relative is affected). As discussed in section 3.1.6, and demonstrated by modeling, the number of affected family members in a family determines the likelihood of a single gene being the cause in that particular family[118]. Analysis of the families involved in this study confirmed this observation, as ALS genes were identified in 41% (5/12) of families with two affected members, and 56% (5/9) of families with three affected members.

A theoretical rate of chance co-occurrence of ALS in a second person in a family was calculated to be 4.1%, based on the lifetime risk to each individual for developing ALS. This theoretical result was confirmed in a family aggregation study, which calculated a background rate of ALS co-occurring by chance among relatives of controls at 4.2%. This study has implications for the utilization of families for gene discovery. Larger families with multiple affected members are a better choice for genetic interrogation.

Criteria for the definition of familial ALS were proposed and the proportion of patients falling into each category was reported in chapter seven (section 6.5.3).
Criteria for Familial Amyotrophic Lateral Sclerosis

**Definite FALS**
- History: Affected patient with **two** first or second degree relatives with ALS
- Genetics: Affected patient with one relative with ALS, and testing positive for an ALS gene

**Probable FALS**
- History: Patient with **one** first or second degree relative with ALS

**Possible FALS**
- History: Patient with one or more third degree relative with ALS

**Suspected FALS**
- History: Patient with one or more affected relative with ALS of fourth degree or greater

**Note:**
1. Patients with a family history of FTD should be recorded and considered for C9orf72 testing
2. Record the size of the kindred and the relationship between the affected individuals

First degree: Parent, sibling, child; Second degree: Grandparent, grandchild, uncle/niece, aunt/nephew; Third degree: First cousin, great aunt etc.

Table 7.1 Proposed criteria for Familial ALS

In chapter three (3.2) the rate of FALS from prospective population-based studies was calculated to be 5.1%, rather than 10%, which was the rate quoted in virtually every epidemiological study of ALS published since 1955.

This aim of determining the true rate of familial ALS was achieved by carrying out a large family aggregation study in a prospective, population-based incident cohort of ALS patients and their families compared to matched controls. Just over 7% of ALS patients reported a family history of ALS, however, upon verification with death certification a further 8% of patients' relatives had been diagnosed with ALS. 16% of ALS patients had a family history of ALS in any degree of relative. When limited to ALS in another first, second- or third-degree relative the rate of FALS was 14%, when limited to first- and second- degree relatives only, the rate was 12%, and when limited to first-degree relatives only the rate was 6%. This study demonstrated two things about self-reporting; firstly the pilot study demonstrated that cognitively impaired patients were unable to give an accurate report of family history themselves, and, secondly, patients and their family members may be unaware of a family history of ALS even when one does exist. This detailed family history method, which included
the procurement of death certificates, doubled the reported rate of familial ALS from 7% to 16% among questionnaire respondents. The use of the proposed criteria for familial ALS make it easy to identify large families who are not known to carry any ALS genes who may be used be used in genetic studies. It is likely that the rate of familial disease is also underreported in other neurodegenerative studies such as PD and AD.

Information from the family aggregation study allowed comparison of the risk of developing ALS among family members of ALS patients compared with relatives of controls. Subgroup analysis revealed that the risk of developing ALS in relatives of patients without the C9orf72 repeat expansion is only double the risk of the general population, which means that the lifetime risk in those people is about one in two hundred. The risk to relatives of ALS patients with the C9orf72 repeat expansion developing ALS is more than thirty times higher than in the general population. Given that 90% of patients with ALS do not carry the repeat expansion, the fact that the lifetime risk of developing ALS among the relatives of the remainder of patients is only marginally increased (to less than 0.5%) may be reassuring for relatives.

Direct comparison of the risk of dementia in relatives of all ALS patients and controls only yielded a marginally increased hazard rate of 1.2, just reaching statistical significance. When comparisons were made between the rate of dementia in controls and relatives of patients with the C9orf72 repeat expansion, the hazard ratio (1.6) for dementia was significantly increased.

The risk of dementia was not significantly increased among the relatives of ALS patients without the repeat expansion. However, when this group was split into those patients with and without cognitive impairment, the relatives of patients with cognitive impairment had a significantly higher burden of dementia (HR 2.6). Relatives of non-C9 ALS patients with abnormal cognition also had a significantly higher rate of familial disease. This information suggests that there may be another gene associated with ALS that causes an increased rate of dementia in ALS patients and their relatives, and is also associated with an increased rate of familial ALS in that cohort. Future genetic studies should consider the inclusion of non-C9
cognitively impaired ALS patients, as they may be associated with a single disease-causing gene. This group warrants a detailed neuropsychological assessment to determine if the pattern of dementia is similar to that seen with C9orf72.

The rate of schizophrenia and suicide were increased in relatives of all ALS cases compared to controls with a hazard rate of 4.2, and 4.9 respectively. When subgroup analysis was carried out to compare relatives of patients with and without the C9orf72 repeat expansion to relatives of controls, the rate of schizophrenia and suicide was increased in both ALS groups, but the rate of suicide was significantly higher in the relatives of C9 ALS patients compared to relatives without the repeat expansion. This observation has never been demonstrated before and will open up avenues of research into the links between ALS, FTD and schizophrenia. Future areas of research could include neuropsychological testing, including measurements of impulsivity, to identify endophenotypes among ALS patients and their relatives. Careful characterization of ALS patients who report a family history of schizophrenia is required, including examination of first- and second-degree relatives. Resting state EEG studies may also be useful in characterizing a signature in ALS patients with increased burden of neuropsychiatric disease.

The other question generated by this research is the potential use of antipsychotics as neuroprotective agents in patients at risk of developing ALS or in patients with ALS. The literature on this topic is sparse and future studies could focus on determining whether patients on antipsychotic medication do in fact develop ALS less often, when corrected for factors such as decreased life expectancy and competing cause of death. If there is indeed a common pathway for the development of ALS and schizophrenia then antipsychotic medications may potentially be of benefit in ALS.

7.1.2 Epidemiology

Epidemiological analysis revealed that the corrected incidence of ALS remained the same throughout the study. There was a trend toward an increase in age of onset, but this was not confirmed statistically. There was no difference in survival between the different time periods despite the introduction of a number of interventions. However, a survival benefit of
access to an ALS multidisciplinary clinic was demonstrated. A direct comparison between two separate registers confirmed that the corrected incidence is the same, indicating excellent capture in both regions. Survival time in Northern Ireland is shorter than in the Republic of Ireland, and while this may be due, in part, to the existence of an ALS multidisciplinary clinic in the Republic of Ireland, it does not entirely account for the disparity. The rate of familial ALS increased between the former two time periods, and the latter two, in the Irish population. This is likely to reflect better reporting of family history. The age of onset of familial ALS is younger than in sporadic cases.

Two subgroups of familial ALS were identified using epidemiological data; the former group, now known to carry the C9orf72 repeat expansion, has more cognitive impairment and shorter survival, compared to the latter. This discovery of two subgroups was made before the identification of the repeat expansion, and highlights the usefulness of a register in identifying subgroups that can inform genetic studies.

Future work in the field of epidemiology should include the use of splines to compare survival, as well as validating prognostic indicators that have been proposed based on other ALS registries[35].

7.1.3 Characterization of C9orf72 phenotype

ALS patients with an expanded hexanucleotide repeat in C9orf72 represent a recognizable subphenotype characterised by a lower age of onset, the presence of cognitive and behavioural impairment, specific neuroimaging changes, a strong family history of ALS or FTD, and reduced survival. A predictive algorithm was created to aid in the decision of which patients to test for the gene. Fecundity also appeared to be reduced among women carrying the C9orf72 repeat expansion.

Much future work is necessary on C9orf72-associated ALS. Firstly, the cut-off point for a pathogenic repeat number needs to be clarified. In this study we choose a repeat number of 24 as the cut-off figure, however the two patients had 22 repeats and they both had a younger age of onset, a family history of FTD, both had behavioural impairment, and one had a strong family history of psychiatric disease. Certainly, these two patients with 22 repeats have a
phenotype very similar to those patients with a \textit{C9orf72} repeat number greater than 24. It is likely that there are other genetic factors influencing the onset of disease in patients with the repeat expansion. None of the patients in this cohort testing positive for \textit{C9orf72} had another known gene, but a number of studies have reported the presence of other mutations in patients with the \textit{C9orf72} repeat expansion and ALS.

A much larger number of patients is needed to stratify ALS patients according to repeat size with the purpose of identifying any correlations.

A large study that has the primary aim of establishing fecundity in ALS is needed. If the findings are replicated, DNA samples from patients with absolute or limited fertility could be used in studies of genetic variants.

Future work is needed to validate the algorithm that is used to aid in prediction of which ALS patients necessitate genetic testing for \textit{C9orf72}.

\textbf{7.2 Using information generated to direct future genetic studies}

One of the aims outlined in the PhD introduction was to use data collected from the register, and family aggregation data generated, to choose appropriate candidates for exploratory gene discovery projects using exome sequencing techniques or alternative approaches.

I have already demonstrated the success of utilizing epidemiological data to identify a subgroup of patients with familial ALS and cognitive impairment. This group was subsequently identified as the group carrying the \textit{C9orf72} repeat expansion.

\textbf{Familial ALS subgroup:} Results from the epidemiology study in chapter 5, and the identification of the \textit{C9orf72} phenotype in chapter 6, demonstrate that there is a cohort of familial cases with prolonged survival compared to other patients with ALS. They may carry a gene associated with better prognosis.

\textbf{Specific familial cases – Families with ALS and FTD, from a particular region:} In the chapter on epidemiology, the rate of familial ALS was determined to be higher in patients from County Tipperary. Kindreds from this region, as outlined in section 6.8.6, have multiple affected family members with ALS, FTD, and other evidence of psychiatric disease, suggesting
that a founder effect may be specific to a common ancestor from this region. DNA samples from the family described in section 6.8.6 have been selected for an exome sequencing project.

**Specific familial cases – Families with ALS and other neurodegenerative diseases, with a particular surname:** In the chapter on epidemiology, data from the register was used to identify one surname that was over represented among Irish ALS patients. In section 6.8.6, I described the largest kindred discovered through the family aggregation project. This family carried the name that was most over-represented on the register. DNA samples from this family have been selected for an exome sequencing project.

**Impaired fecundity:** Limited fecundity has been demonstrated in ALS patients compared to controls, and in particular in the female ALS patients carrying the repeat expansion. Future work may focus on the identification of genetic structural variants or other repeat expansions in non-C9 ALS patients with limited fertility.

**Cognitively impaired non-C9 patients:** It has been demonstrated that cognitively impaired non-C9 ALS patients are statistically more likely to have a family history of ALS, and a higher burden of dementia among relatives. These findings provide evidence for the presence of a single gene driving this specific phenotype.

**Presence of psychiatric illness within relatives of ALS patients:** Patients with ALS have a higher rate of schizophrenia and suicide among family members than among relatives of controls. This is the first association study reporting a connection between ALS and psychiatric disease. Further investigation of the putative pathways and specific subphenotypes in families containing members with ALS and schizophrenia is required before genetic studies could be considered.

### 7.3 Conclusion

This thesis has made significant contributions to the current literature on ALS in the fields of epidemiology, familial ALS and aggregation studies, and the phenotype of ALS associated with the C9orf72 repeat expansion. Subgroups identified in the course of this thesis will be pursued in future genetic studies.


Appendix A Questionnaire for consensus of definition study

Defining Familial Amyotrophic Lateral Sclerosis

Thank you for taking ten minutes of your time to answer some questions about your clinical views on the definition of Familial Amyotrophic Lateral Sclerosis (FALS). There are no correct answers. We are seeking to build a consensus. All questionnaires are confidential and you will not be asked for your name. Your views, as a researcher and clinician, are much valued.

Kind regards,
Susan Byrne
(HRF research fellow, Trinity College, Ireland.)
suabyrne@gmail.com

Demographic Information

Sex: Male ☐ Female ☐ Country of origin: __________________________

What is your clinical role?
Trainee Neurologist ☐
Neurologist ☐
Clinical Geneticist ☐

Do you have a special interest in ALS?
Yes ☐ No ☐

If yes, for how many years have you been specializing in the field of ALS?
<5 years ☐ 5-10 years ☐ 10-15 years ☐ 15-20 years ☐ >20 years ☐

If you are an ALS neurologist what are your research interests? (check box for more than one)
ALS epidemiology ☐ Genetics ☐ Familial ALS ☐
ALS clinical trials ☐ ALS cognition ☐ Other ☐

Page 1 - Irish Motor Neuron Disease Registry

252
Q1: Do you think there is a standard definition among neurologists for FALS?
Yes □  No □  No opinion □

Q2: When you read the literature and see FALS mentioned in a paper, what definition for FALS do you assume the authors are using? (please tick one answer only)
i. A patient with ALS who has one first degree relative affected with ALS □
ii. A patient with ALS who has two or more first degree relatives affected with ALS □
iii. A patient with ALS with either one first or second degree relative with ALS □
iv. A patient with ALS with any relative with ALS, no matter how distant □
v. A patient with sporadic ALS who tests positive for a known ALS gene □
(e.g. SOD1, FUS, TARDBP etc)
Note: 1st degree relative: Parent, Sibling, Child
2nd degree relative: Grandparent, Aunt/Uncle, Niece/Nephew

Q3: What do you consider to be the rate of FALS?
0-4% □  5% □  5-10% □
10% □  10-15% □  >15% □

Q4: Do any of the definitions below match the definition you use for FALS? If yes, please tick the one that applies:
i. A patient with ALS who has only one first degree relative affected with ALS □
ii. A patient with ALS who has two or more first degree relatives affected with ALS □
iii. A patient with ALS with either one first or second degree relative with ALS □
iv. A patient with ALS with any relative with ALS, no matter how distant □
v. A patient with sporadic ALS who tests positive for a known ALS gene □
Q5: Please outline, in your own words, the definition for FALS that you use in your clinical practice:

Q6: Do you routinely refer patients with FALS for genetic counseling?
- Yes ☐
- No ☐
- No opinion ☐

Q7: Do you routinely do genetic testing on patients with a family history of ALS?
- Yes ☐
- No ☐
- No opinion ☐

Q8: Do you routinely do genetic testing on patients with no family history of ALS?
- Yes ☐
- No ☐
- No opinion ☐

Q9: Do you think that patients with familial ALS are more likely to have cognitive impairment than patients with sporadic ALS?
- Yes ☐
- No ☐
- No opinion ☐
Q10: If a patient presented to your clinic with the following family history, would you record them as having FALS?
Please do not refer to your previous answers. Please answer each scenario individually and feel free to add additional comments.

Additional Comments:

Yes ☐
No ☐
Maybe ☐

Additional Comments:

Yes ☐
No ☐
Maybe ☐

Additional Comments:

Yes ☐
No ☐
Maybe ☐

Additional Comments:

Yes ☐
No ☐
Maybe ☐

Additional Comments:

Yes ☐
No ☐
Maybe ☐

Filled in squares/circles represent a person who has ALS. Your patient is annotated with the green arrow.
Q11: The follow criteria have been proposed for FALS:

<table>
<thead>
<tr>
<th>Proposed Criteria for Familial Amyotrophic Lateral Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definite FALS</strong></td>
</tr>
<tr>
<td>History: ALS patient with at least two 1st or 2nd degree relatives with ALS</td>
</tr>
<tr>
<td>Genetics: ALS patient with at least one relative with ALS and gene positive co-segregation</td>
</tr>
<tr>
<td><strong>Probable FALS</strong></td>
</tr>
<tr>
<td>History: ALS patient with one 1st or 2nd degree relative with ALS</td>
</tr>
<tr>
<td><strong>Possible FALS</strong></td>
</tr>
<tr>
<td>History: ALS patient with a distant relative with ALS (more distant than 1st or 2nd degree)</td>
</tr>
<tr>
<td>Genetics: Sporadic ALS patient with no family history, but positive for a FALS gene</td>
</tr>
<tr>
<td>Neuroradiochemistry: ALS patient with a family member with confirmed fronto-temporal dementia</td>
</tr>
</tbody>
</table>

Do you have any comments to make on these criteria?

Q12: Do you think there should be a consensus meeting which sets out with the aim of defining FALS?

Yes □  No □  No opinion □

Thank you very much for taking the time to complete this questionnaire. Please feel free to add any additional comments. Add your email address if you would like feedback on this survey.
SECTION I. INFORMATION ABOUT THE PROBAND

Name: _________________________________________________
    (Last) (Maiden) (First) Middle

Address: ________________________________________________

Birth date: ________________________________ Sex: Male ___ / Female ___
    (Day/Month/Year)

Phone: ________________________________________________

What is the proband's diagnosis? __________________________

When did symptoms first appear and what were the first symptoms?

Who made the diagnosis? Please give doctor's name, address and phone #: ________________________________

When was the diagnosis made? ________________________________

Who is the primary physician now? Please give doctor's name, address and phone #: ________________________________

Are there other family members with a similar illness? ________________________________

What was the individual's occupation? ________________________________

What was your address of your residence at the time ALS was diagnosed? ________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)

Approval date 16th June 2005
Family History Questionnaire, Beaumont Hospital, Dublin.

Which of the following best describes your racial background?

<table>
<thead>
<tr>
<th>White/Caucasian</th>
<th>North American Indian, Eskimo/Aleutian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oriental/Asian</td>
<td>Black/African American</td>
</tr>
<tr>
<td>Hawaiian, Pacific Islander</td>
<td>Other</td>
</tr>
</tbody>
</table>

Are you of Spanish or Hispanic origin or ancestry? ________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)

Approval date 16th June 2005
SECTION II. PROBAND'S SPOUSE AND CHILDREN OLDER THAN 18 YEARS OF AGE

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems? Explain

______________________________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc? Explain

______________________________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

______________________________________________________________

______________________________________________________________

Do any of these children have any health problems?

______________________________________________________________

Were there any miscarriages or stillborns within this family? Specify:

______________________________________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND) Approval date 16th June 2005
### SECTION III. PROBAND'S PARENTS

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What is the racial background of your Mother? 

What is the racial background of your Father? 

What was your mother’s place of death? 

What was your father’s place of death? 

What is the address of your parent(s) if living? 

Do/did either of these people have any health problems? 

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc? 

Explain
**SECTION IV. PROBAND'S SIBLINGS**

**SIBLING ONE**

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems? Explain ____________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc? Explain ____________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

Were there any miscarriages or stillborns within this family? Specify: ____________________________________________

6

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND) Approval date 16th June 2005
### SECTION IV. PROBAND’S SIBLINGS (continued)

#### SIBLING TWO

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Birthdate</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write “N/A” if the question is not applicable i.e. no medical conditions. Also please write “No Information Available” for sections where little information is known.

Do/did any of these people have any health problems?  
Explain

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc?  
Explain

Do any of these children have children?  (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

Were there any miscarriages or stillborns within this family? Specify:

7

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

“Proband” is the patient (i.e. the individual with MND)
### SIBLING THREE

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems? 
**Explain**

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc? 
**Explain**

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.) 

Do any of these children have any health problems? 

Were there any miscarriages or stillborns within this family? Specify: 

**DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE**

"Proband" is the patient (i.e. the individual with MND) 

Approval date 16\textsuperscript{th} June 2005
### SIBLING FOUR

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

**Do/did any of these people have any health problems?**

Explain ____________________________________________________________

**Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc?**

Explain ____________________________________________________________

**Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)**

______________________________________________________________

**Do any of these children have any health problems?**

______________________________________________________________

**Were there any miscarriages or stillborns within this family? Specify:**

______________________________________________________________

**DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE**

“Proband” is the patient (i.e. the individual with MND)
Does the proband have any half-brothers or sisters not parented by the two people listed in Section III? Please list the two parents, their birthdates, dates of death, and the names, birthdates, and dates of deaths of the half-brothers/sisters:

E. Please provide the same information for any additional siblings.

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
**SECTION V. PARENTS OF PROBAND’S MOTHER (maternal grandparents)**

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What is the racial background of the mother?  
What is the racial background of the father?  
What is the mother’s place of death?  
What is the father’s place of death?  
Do/did either of these people have any health problems?  

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc?  
Explain

---

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)  
Approval date 16th June 2005
SECTION VI. SIBLINGS OF PROBAND'S MOTHER (maternal aunts and uncles)

<table>
<thead>
<tr>
<th>SIBLING ONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last Name (Maiden) First</td>
</tr>
<tr>
<td>Brother/Sister:</td>
</tr>
<tr>
<td>Spouse:</td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
<tr>
<td>3.</td>
</tr>
<tr>
<td>4.</td>
</tr>
<tr>
<td>5.</td>
</tr>
<tr>
<td>6.</td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems?
Explain

Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc?
Explain

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

Were there any miscarriages or stillborns within this family? Specify:

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
**SECTION VI. SIBLINGS OF PROBAND’S MOTHER (maternal aunts and uncles) (continued)**

<table>
<thead>
<tr>
<th>SIBLING TWO</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Last Name (Maiden) First</strong></td>
<td><strong>Sex M/F</strong></td>
</tr>
<tr>
<td>Brother/Sister:</td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write “N/A” if the question is not applicable i.e. no medical conditions. Also please write “No Information Available” for sections where little information is known.

Do/did any of these people have any health problems? 
Explain ________________________________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc? 
Explain ________________________________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems? 

Were there any miscarriages or stillborns within this family? Specify:

**DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE**

“Proband” is the patient (i.e. the individual with MND)
SECTION VI. SIBLINGS OF PROBAND’S MOTHER (maternal aunts and uncles) (continued)

SIBLING THREE

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems?
Explain ____________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc?
Explain ____________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

Were there any miscarriages or stillborns within this family? Specify:

15

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)

Approval date 16th June 2005
SECTION VI. SIBLINGS OF PROBAND’S MOTHER (maternal aunts and uncles) (continued)

<table>
<thead>
<tr>
<th>SIBLING FOUR</th>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write “N/A” if the question is not applicable i.e. no medical conditions. Also please write “No Information Available” for sections where little information is known.

Do/did any of these people have any health problems? Explain ____________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc? Explain ____________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.) ____________________________________________

Do any of these children have any health problems? ____________________________________________

Were there any miscarriages or stillbirths within this family? Specify: ____________________________________________

16

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

“Proband” is the patient (i.e. the individual with MND) Approval date 16th June 2005
SECTION VI. SIBLINGS OF PROBAND'S MOTHER (maternal aunts and uncles) (continued)

Does the probands' mother have any half-brothers or sisters not parented by the two people listed in Section V? Please list the two parents, their birthdates, dates of death, and the names, birthdates, and dates of deaths of the half-brothers/sisters:

E. Please provide above information for any additional siblings.

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
### SECTION VII. PARENTS OF PROBAND'S FATHER (Paternal Grandparents)

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Birthdate</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

What is the racial background of the mother? 

What is the racial background of the father?

What is the mother's place of death?

What is the father's place of death?

Do/did either of these people have any health problems?

Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc?

Explain

---

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)

Approval date 16th June 2005
SECTION VIII. SIBLINGS OF PROBAND’S FATHER (paternal aunts and uncles) (continued)

SIBLING ONE

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do/did any of these people have any health problems? Explain ________________________________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc? Explain ________________________________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.) ________________________________________________________________

Do any of these children have any health problems? Explain ________________________________________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND) Approval date 16th June 2005
SECTION VIII. SIBLINGS OF PROBAND'S FATHER (paternal aunts and uncles) (continued)

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do/did any of these people have any health problems?  
Explain ______________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc?  
Explain ___________________________________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents.  Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)  
Approval date 16th June 2005
**SECTION VIII. SIBLINGS OF PROBAND’S FATHER (paternal aunts and uncles) (continued)**

**SIBLING THREE**

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.

Do/did any of these people have any health problems? Explain ____________________________________________________________________________

Do/did any of these people have any neurological problems, such as Dementia, Parkinson’s disease, Multiple Sclerosis etc? Explain ____________________________________________________________________________

Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

Do any of these children have any health problems?

Were there any miscarriages or stillbirths within this family? Specify: ____________________________________________________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND) Approval date 16th June 2005
**SECTION VIII. SIBLINGS OF PROBAND'S FATHER** *(paternal aunts and uncles) (continued)*

**SIBLING FOUR**

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Birthdate Date/Place/City/Country</th>
<th>Date of Death</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: When specifying an illness please indicate the individual to whom it refers by either name or the number where they appear on the above list. Also please write "N/A" if the question is not applicable i.e. no medical conditions. Also please write "No Information Available" for sections where little information is known.*

1. Do/did any of these people have any health problems? Explain _______________________________________________________________________

2. Do/did any of these people have any neurological problems, such as Dementia, Parkinson's disease, Multiple Sclerosis etc? Explain _______________________________________________________________________

3. Do any of these children have children? (Please list separately with dates of birth and parents. Do not include information on children less than 18 years of age.)

4. Do any of these children have any health problems?

5. Were there any miscarriages or stillborns within this family? Specify:

*DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE*

"Proband" is the patient (i.e. the individual with MND) Approval date 16th June 2005
SECTION VIII. SIBLINGS OF PROBAND'S FATHER (paternal aunts and uncles) (continued)

Does the proband's mother have any half-brothers or sisters not parented by the two people listed in Section V? Please list the two parents, their birthdates, dates of death, and the names, birthdates, and dates of deaths of the half-brothers/sisters:

E. Please provide above information for any additional siblings on a separate sheet.
Family History Questionnaire – Patient

Thank you very much for taking the time to fill out this questionnaire. We appreciate the effort that you and your family are taking to help with ongoing research into Motor Neuron Disease. If you have any questions or would prefer to carry out this questionnaire by telephone please contact x.

Here are a number of points to make filling in this form easier:

- Questions are asked about your children over the age of 18, your parents, your brothers and sisters, your aunts and uncles and your grandparents.
- We do not need the names of anyone who is alive. For people who are alive their sex (either male or female), their age, and whether they are well or not is enough information.
- For relatives who have passed away we endeavor to source death certificates. This helps in verification of the history. In order to get a death certificate it is very helpful to have the name of the person, their age at death, the date or year they died and their place of death.
- All information is confidential.

We thank you again for your participation in research.

Best wishes & thanks,
Susan Byrne

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

“Proband” is the patient (i.e. the individual with MND)
Section 1: Demographic information:

Name: ______________________ * Maiden name (if applicable): ______________________

(please note that the word proband refers to the person whose name is entered here)

Address: ______________________
Birth date: ___________ Phone: ___________
Occupation: ______________________

Date of completion of questionnaire: ______________________
Names of family members helping to complete questionnaire: ______________________

Section 2: Information on diagnosis:

What is the proband’s diagnosis: ______________________
On what date did symptoms first appear: ______________________
What were the first symptoms: ______________________
On what date was the diagnosis made: ______________________
Please give the name of the neurologist who made the diagnosis: ______________________
Are there other family members with a similar illness: ______________________
Section 3: Information about your family:

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify • date/year of death • place of death • cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Section 4: Information about your parents:

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify • date/year of death • place of death • cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOTHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FATHER:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
### Section 5: Information about your brothers and sisters (siblings)

<table>
<thead>
<tr>
<th>Sibling one</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sibling two</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
### Section 5: Information about your brothers and sisters (siblings)

<table>
<thead>
<tr>
<th>Sibling three</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify • date/year of death • place of death • cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sibling four</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify • date/year of death • place of death • cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
**Section 5: Information about your brothers and sisters (siblings)**

<table>
<thead>
<tr>
<th>Sibling five</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify • date/year of death • place of death • cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brother/Sister:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If you have any more brothers or sister please provide information.

__________________________________________________________________________

If you have any half-brothers or half-sisters please provide information.

__________________________________________________________________________

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
Section 6: Information about your maternal grandparents (your mother's parents)

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Grandmother:  

Grandfather:

Section 7: Information about your maternal aunts and uncles (siblings of your mother)

<table>
<thead>
<tr>
<th>Aunt/Uncle One</th>
<th>Sex M/F</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle:  

Spouse:

Children over 18 years of age:  

1.  

2.  

3.  

4.  

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
### Section 7: Information on your maternal aunts and uncles (siblings of your mother)

<table>
<thead>
<tr>
<th>Aunt/Uncle Two</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle: |
Spouse: |
Children over 18 years of age: |
1. |
2. |
3. |
4. |

<table>
<thead>
<tr>
<th>Aunt/Uncle Three</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle: |
Spouse: |
Children over 18 years of age: |
1. |
2. |
3. |
4. |

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
Section 7: Information on your maternal aunts and uncles (siblings of your mother)

<table>
<thead>
<tr>
<th>Aunt/Uncle Four</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle:  
Spouse:  
Children over 18 years of age:  
1.  
2.  
3.  
4.  

<table>
<thead>
<tr>
<th>Aunt/Uncle Five</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle:  
Spouse:  
Children over 18 years of age:  
1.  
2.  
3.  
4.  

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE  

"Proband" is the patient (i.e. the individual with MND)  

Version 3 22/03/2011
Section 8: Information about your paternal grandparents (your father’s parents)

<table>
<thead>
<tr>
<th>Last Name (Maiden) First</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Grandmother:  
Grandfather:

Section 9: Information about your paternal aunts and uncles (siblings of your father)

<table>
<thead>
<tr>
<th>Aunt/Uncle One</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
</tbody>
</table>

Aunt/Uncle:  
Spouse:  
Children over 18 years of age:
1.  
2.  
3.  
4.  

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
**Section 9: Information about your paternal aunts and uncles (siblings of your father)**

<table>
<thead>
<tr>
<th>Aunt/Uncle Two</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aunt/Uncle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Children over 18 years of age:**

1.  
2.  
3.  
4.  

<table>
<thead>
<tr>
<th>Aunt/Uncle Three</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aunt/Uncle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Children over 18 years of age:**

1.  
2.  
3.  
4.  

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)

Version 3 22/03/2011
Section 9: Information about your paternal aunts and uncles (siblings of your father)

<table>
<thead>
<tr>
<th>Aunt/Uncle Four:</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td>Aunt/Uncle:</td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aunt/Uncle Five:</th>
<th>Sex</th>
<th>Year and county of birth</th>
<th>Please specify any health problems (including problems such as dementia, parkinsons disease etc)</th>
<th>If deceased, please specify</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td>date/year of death</td>
</tr>
<tr>
<td>Aunt/Uncle:</td>
<td></td>
<td></td>
<td></td>
<td>place of death</td>
</tr>
<tr>
<td>Spouse:</td>
<td></td>
<td></td>
<td></td>
<td>cause of death</td>
</tr>
<tr>
<td>Children over 18 years of age:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
If there is any information that we did not ask you for, that you would like to share please feel free to include it below. If you have any cousins / distant relatives that you would like to mention this would also be helpful:

________________________________________________________

________________________________________________________

If you would like to make any general comments please feel free to do this:

________________________________________________________

________________________________________________________

Many thanks again for taking the time to complete this questionnaire. A stamped addressed envelope is provided for you to return this form.

Best wishes,

Susan Byrne
Research Doctor, Beaumont Hospital

13

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with MND)
## Appendix D Specific causes of death

<table>
<thead>
<tr>
<th>Cause</th>
<th>Relative of case</th>
<th>Relative of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>$n=1598$</td>
<td>$n=2341$</td>
</tr>
<tr>
<td>Unspecified Cardiac</td>
<td>Count</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>5.9%</td>
</tr>
<tr>
<td>Old Age</td>
<td>Count</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>7.4%</td>
</tr>
<tr>
<td>MI</td>
<td>Count</td>
<td>252</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>15.8%</td>
</tr>
<tr>
<td>CCF</td>
<td>Count</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>4.0%</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Count</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>1.6%</td>
</tr>
<tr>
<td>Alzheimers</td>
<td>Count</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>.7%</td>
</tr>
<tr>
<td>Parkinsons disease</td>
<td>Count</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>.6%</td>
</tr>
<tr>
<td>C2H5OH related</td>
<td>Count</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>.7%</td>
</tr>
<tr>
<td>Accident</td>
<td>Count</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>3.0%</td>
</tr>
<tr>
<td>perinatal cause (under 5yrs)</td>
<td>Count</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>2.6%</td>
</tr>
<tr>
<td>TB</td>
<td>Count</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>2.3%</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Count</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>4.0%</td>
</tr>
<tr>
<td>COPD</td>
<td>Count</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>2.5%</td>
</tr>
<tr>
<td>Subarachnoid haemmorhage</td>
<td>Count</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>.7%</td>
</tr>
<tr>
<td>No Information available</td>
<td>Count</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>6.5%</td>
</tr>
<tr>
<td>Appendicitis</td>
<td>Count</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>% within group</td>
<td>.0%</td>
</tr>
<tr>
<td>Condition</td>
<td>Count</td>
<td>% within group</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------</td>
<td>----------------</td>
</tr>
<tr>
<td>Stroke</td>
<td>109</td>
<td>6.8%</td>
</tr>
<tr>
<td>Cancer</td>
<td>341</td>
<td>21.3%</td>
</tr>
<tr>
<td>Dementia, not specified</td>
<td>49</td>
<td>3.1%</td>
</tr>
<tr>
<td>BPAD</td>
<td>2</td>
<td>.1%</td>
</tr>
<tr>
<td>Suicide</td>
<td>14</td>
<td>.9%</td>
</tr>
<tr>
<td>War</td>
<td>4</td>
<td>.3%</td>
</tr>
<tr>
<td>Childbirth</td>
<td>17</td>
<td>1.1%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5</td>
<td>.3%</td>
</tr>
<tr>
<td>Respiratory Problems</td>
<td>14</td>
<td>.9%</td>
</tr>
<tr>
<td>Liver Failure</td>
<td>1</td>
<td>.1%</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>2</td>
<td>.1%</td>
</tr>
<tr>
<td>Deteriorated after broken hip</td>
<td>8</td>
<td>.5%</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>16</td>
<td>1.0%</td>
</tr>
<tr>
<td>Murdered</td>
<td>0</td>
<td>.0%</td>
</tr>
<tr>
<td>MND</td>
<td>26</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

% within group: Stroke 6.8%, Cancer 21.3%, Dementia, not specified 3.1%, BPAD .1%, Suicide .9%, War .3%, Childbirth 1.1%, Diabetes .3%, Respiratory Problems .9%, Liver Failure .1%, Epilepsy .1%, Deteriorated after broken hip .5%, Myocarditis 1.0%, Murdered .0%, MND 1.6%
<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>% within group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debility</td>
<td>1</td>
<td>.1%</td>
</tr>
<tr>
<td>% within group</td>
<td>4</td>
<td>.2%</td>
</tr>
<tr>
<td>Meningitis</td>
<td>4</td>
<td>.3%</td>
</tr>
<tr>
<td>% within group</td>
<td>5</td>
<td>.2%</td>
</tr>
<tr>
<td>Post-surgical complications</td>
<td>0</td>
<td>.0%</td>
</tr>
<tr>
<td>% within group</td>
<td>10</td>
<td>.4%</td>
</tr>
<tr>
<td>Influenza</td>
<td>5</td>
<td>.3%</td>
</tr>
<tr>
<td>% within group</td>
<td>17</td>
<td>.7%</td>
</tr>
<tr>
<td>Gastrointestinal Problems</td>
<td>10</td>
<td>.6%</td>
</tr>
<tr>
<td>% within group</td>
<td>42</td>
<td>1.7%</td>
</tr>
<tr>
<td>Unspecified infection</td>
<td>13</td>
<td>.8%</td>
</tr>
<tr>
<td>% within group</td>
<td>44</td>
<td>1.8%</td>
</tr>
<tr>
<td>Spina Bifida</td>
<td>2</td>
<td>.1%</td>
</tr>
<tr>
<td>% within group</td>
<td>1</td>
<td>.0%</td>
</tr>
<tr>
<td>Pulmonary Embolism</td>
<td>5</td>
<td>.3%</td>
</tr>
<tr>
<td>% within group</td>
<td>15</td>
<td>.6%</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>0</td>
<td>.0%</td>
</tr>
<tr>
<td>% within group</td>
<td>1</td>
<td>.0%</td>
</tr>
<tr>
<td>Phythysis</td>
<td>7</td>
<td>.4%</td>
</tr>
<tr>
<td>% within group</td>
<td>15</td>
<td>.6%</td>
</tr>
<tr>
<td>Rheumatism</td>
<td>7</td>
<td>.4%</td>
</tr>
<tr>
<td>% within group</td>
<td>1</td>
<td>.0%</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>2</td>
<td>.1%</td>
</tr>
<tr>
<td>% within group</td>
<td>2</td>
<td>.1%</td>
</tr>
<tr>
<td>Total</td>
<td>1598</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within group</td>
<td>2431</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Appendix E Published articles arising from this thesis

1. ‘Familial Aggregation in Amyotrophic Lateral Sclerosis’
S Byrne, O Hardiman.
*Annals of Neurology*. April 2010: Vol 67 (4); 554

2. ‘Rate of Familial Amyotrophic Lateral Sclerosis: A Systematic Review and Meta-analysis
S Byrne, C Walsh, C Lynch, P Bede, M Elamin, K Kenna, R McLaughlin, O Hardiman

3. ‘Proposed criteria for Familial Amyotrophic Lateral Sclerosis’
S Byrne, C Walsh, C Lynch, P Bede, M Elamin, K Kenna, R McLaughlin, O Hardiman

4. ‘Absence of Consensus in Diagnostic Criteria for Familial Neurodegenerative Diseases’
S Byrne, M Elamin, P Bede, O Hardiman.

5. ‘Phenotype, genotype and population-based frequency of C9orf72 repeat expansion in ALS’

6. Age at onset of Amyotrophic lateral sclerosis is Proportional to Life Expectancy
S Byrne, I Jordan, M Elamin, O Hardiman.
*Amyotrophic lateral sclerosis and other motor neuron diseases, 2013.*
7. Aggregation of Neurologic and Neuropsychiatric Disease in ALS Kindreds: A population based case controlled cohort study of Familial and Sporadic ALS

S Byrne, M Heverin, M Elamin, P Bede, C Lynch, K Kenna, R MacLaughlin, C Walsh, A Al-Chalabi, O Hardiman.

*Annals of Neurology, 2013*
ANNALS of Neurology

References

DOI: 10.1002/ana.21882

Familial Aggregation in Amyotrophic Lateral Sclerosis
Susan Byrne, MRCP, and Orla Hardiman, FRCP, FAAN

We read with interest a recent article in Annals by Fang and colleagues1 on the relative risk for amyotrophic lateral sclerosis (ALS) in siblings and children of ALS patients and seek to make some comments.

Although the case ascertainment of patients with ALS (probands) in this study is comprehensive, the difficulty in identifying siblings cannot be overlooked. A total of 9,457 Swedish patients with ALS were identified retrospectively using an inpatient register and death certificates. A total of 6,671 probands then had siblings, children, and spouses identified using the Swedish Multi-Generational Register. Only 1,909 identified siblings were included in analysis. This represents identification of only one sibling for every three patients with ALS, and the authors acknowledge this by stating that they could identify siblings for only 20% of the ALS cases who were born since 1932 and had identifiable parents. Nine of the 1,909 identified siblings identified then experienced development of ALS. In this study, it is reported that there is a 17-fold risk for development of ALS among siblings. As ALS diagnoses were identified only through inpatient hospitalization or death certification, it is more likely that siblings with ALS were picked up than those who remained healthy because they would not be included even if inpatient register or death certification. Of 6,671 ALS cases, only 46 relatives with ALS were identified, which gives a rate of familial ALS of only 0.7% in Sweden among siblings and children.

The purpose of a familial aggregation study is two-fold. First, it allows provision of answers to relatives who have questions regarding hereditability of certain conditions. Second, it allows genetic epidemiologists to analyze inheritance patterns with the specific aim of localizing genes or gene clusters in conditions presumed to have a major genetic role. A purely epidemiological study with small numbers of siblings identified and without consideration of disease phenotype among proband-sibling pairs may misrepresent the relative risk among siblings.

To identify the rate of familial ALS and familial aggregation, a study needs to be conducted in a region where a prospective population-based ALS register is in place, where DNA is banked, and where family history information can be collected on all parents, siblings, and children of patients with ALS, as well as second-degree relatives such as aunts, uncles, and cousins.

Department of Neurology, Beaumont Hospital, and Trinity College, Institute of Neuroscience, Dublin, Ireland

Potential Conflicts of Interest
Nothing to report.

Reference

DOI: 10.1002/ana.21883

SWCA Variants and Multiple System Atrophy
Ji Y. Yun, MD1,2, Woon-Woo Lee, MD3, Jee-Young Lee, MD3, Hye J. Kim, MD3, Sung S. Park, MD, PhD, and Beom S. Jeon, MD, PhD3

Multiple system atrophy (MSA) shares the common pathological feature of alpha-synuclein inclusions with Parkinson’s disease (PD). Single nucleotide polymorphism (SNP) of the alpha-synuclein gene (SNCA) is reported to be one of the most reproducible risk factors for PD. Scholz et al.1 recently found an increased risk of SWCA variants in Caucasian MSA patients. They reported that the SNP n11931074 had highly significant associations with an increased risk of the development of MSA (odds ratio = 6.2). The result was subsequently replicated in an independent set of autopsy-proven MSA.2 The frequency of the risk allele T of n11931074 is low, at 2% to 10% in European populations, but is very high, at 51% to 58%, in Asian populations.3

In the present study, we wanted to examine the result in Korean patients with MSA where the risk-allele frequency is high in control population.

A sample size of 100 patient-control pairs was calculated under the assumption of an expected odds ratio (OR) of 2.5, which corresponded to the lower limit of significant ORs (range, 2.7–11.1) in the screening stage reported by Scholz et al.1 One hundred MSA cases were selected from the entries in the Seoul National University Hospital Movement Disorder Database that met the criteria of probable and possible MSA.4 The onset age of MSA was 55.9 ± 7.8 years. Controls were spouses of PD patients who themselves had no family history of parkinsonism, and they were aged 62.5 ± 9.5 years.

The frequency of the risk allele T of n11931074 was 58% in both our MSA and control populations, resulting in an OR of 0.92 under a recessive model (p = 0.77) (Table). This result is in odds with the previous reports.1,2 There were major differences between our study and the previous studies in the ethnicities of the subjects (Korean vs. Caucasian) and the risk-allele frequencies in control populations (58% vs. 8%). Studies involv-
Rate of familial amyotrophic lateral sclerosis: a systematic review and meta-analysis

Susan Byrne,1,3 Cathal Walsh,2 Catherine Lynch,1 Peter Bede,1,3 Marwa Elamin,1,3 Kevin Kenna,2 Russell McLaughlin,3 Orla Hardiman1,3

ABSTRACT

Background The population rate of familial amyotrophic lateral sclerosis (FALS) is frequently reported as 10%. However, a systematic review and meta-analysis of the true population based frequency of FALS has never been performed.

Method A Medline literature review identified all original articles reporting a rate of FALS. Studies were grouped according to the type of data presented and examined for sources of case ascertainment. A systematic review and meta-analysis of reported rates of FALS was then conducted to facilitate comparison between studies and calculate a pooled rate of FALS.

Results 38 papers reported a rate of FALS. Thirty-three papers were included in analysis and the rate of FALS for all studies was 4.6% (95% CI 3.9% to 5.5%). Restricting the analysis to prospective population based registry data revealed a rate of 5.1% (95% CI 4.1% to 6.1%). The incidence of FALS was lower in southern Europe. There was no correlation between rate of FALS and reported SOD1 mutation rates.

Conclusion The rate of FALS among prospective population based registries is 5.1% (CI 4.1 to 6.1%), and not 10% as is often stated. Further detailed prospective population based studies of familial ALS are required to confirm this rate.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease which was first described by Charcot in the mid-nineteenth century. It is predominantly a sporadic disease but a small percentage of cases occur within kindreds.1

In 1955, Kurland et al published a case series of 58 patients in which 10% reported a family history of ALS.2 Although subsequent publications have reported varying rates of familial amyotrophic lateral sclerosis (FALS), the figure of 10% remains as the accepted population frequency of FALS.2–7

There is no definitive definition for FALS. It is generally accepted that the presence of ALS in either a first or second degree relative of the index case constitutes the familial form of the disease. Genes known to be associated with FALS include SOD1, TARDBP, FUS and ANG.5 The frequency of SOD1 mutations has been estimated to account for 10–20% of all FALS cases.6 This estimate is not population based and most studies preferentially recruit patients with familial ALS.6,7 To date only one population based estimate of SOD1 frequency has been carried out, reporting a rate of 13.6.11

Estimation of the rates of SOD1 related FALS is also confounded by the occurrence of mutations in up to 5% of the sporadic population, some of whom represent familial disease with incomplete penetrance.12 The population frequency of the other known causative genes within populations has not been established.

We performed a systematic review and meta-analysis of all studies that presented original data reporting a rate of FALS (ie, the proportion of familial cases among all ALS cases, either in a defined population or in a case series). Analysis of the population based frequency of FALS was then undertaken and, where possible, a geographic comparison was made with the frequency of known SOD1 mutations.

METHODS

Systematic search

A Medline literature search was performed to identify all published studies on FALS, in addition to ALS incidence and prevalence studies from 1966 to October 2009. The MeSH terms 'ALS', 'amyotrophic lateral sclerosis', 'FALS', 'familial amyotrophic lateral sclerosis', 'familial motor neuron(e) disease', 'motor neuron(e) disease', 'MND', 'incidence', 'prevalence' and 'mortality' were used. Additional references were sought from cited articles. Where no information was reported on the rate of FALS in a population based study, the corresponding author was contacted where possible. Unpublished up to date data from the Irish ALS prospective population based register was also used.

Eligibility criteria and data collection

All studies presenting original data that reported a rate of FALS (ie, the proportion of familial cases within a defined cohort) were included in the systematic review. Only studies that demonstrated complete enrolment, either in the form of population based registry or in sequential case series, were analysed. Studies with non-random enrolment or cohorts enriched for familial disease were not included in analysis.

Studies fulfilling inclusion criteria were grouped together according to the type of data presented: (1) prospective registry based studies that aim to capture all cases within a given geographic region in order to define incidence and prevalence; (2) retrospective studies that attempt to capture all cases in a given geographic region with the aim of estimating incidence and prevalence; (3) prospective cases series; and (4) retrospective case series.
### Table 1: Comparison of the type of study, rate of familial amyotrophic lateral sclerosis, source of case ascertainment and case inclusion criteria for each study

<table>
<thead>
<tr>
<th>Country</th>
<th>Years</th>
<th>FALS Total ALS</th>
<th>% FALS</th>
<th>Ascertainment</th>
<th>Diagnosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1: prospective population based registry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Puglia, Southern Italy</td>
<td>1998-1999</td>
<td>2</td>
<td>130</td>
<td>1.50</td>
<td>A, B, C, D, G, K</td>
<td>Bi, H, J, L, N</td>
</tr>
<tr>
<td>Piemont, Northern Italy</td>
<td>1995-2004</td>
<td>53</td>
<td>1280</td>
<td>4.20</td>
<td>A, D, H</td>
<td>Bi, N</td>
</tr>
<tr>
<td>Uruguay</td>
<td>2002-2003</td>
<td>6</td>
<td>143</td>
<td>4.20</td>
<td>A, B, H, J</td>
<td>Bi, I, K</td>
</tr>
<tr>
<td><strong>Group 2: retrospective population based study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>1985-2006</td>
<td>10</td>
<td>244</td>
<td>4.10</td>
<td>B, C, D, L</td>
<td>Bi, G, I, K</td>
</tr>
<tr>
<td>Rochester, USA</td>
<td>1975-1998</td>
<td>37</td>
<td>143</td>
<td>2.80</td>
<td>C, H, J, L</td>
<td>B</td>
</tr>
<tr>
<td>Modena, Italy</td>
<td>1989-1999</td>
<td>4</td>
<td>143</td>
<td>5.00</td>
<td>C, D</td>
<td>D</td>
</tr>
<tr>
<td>Jefferson County, USA</td>
<td>1988-2002</td>
<td>2</td>
<td>36</td>
<td>5.50</td>
<td>C, D, G, H</td>
<td>Bi, F, I, M</td>
</tr>
<tr>
<td>Middle Finland</td>
<td>1976-1981</td>
<td>5</td>
<td>43</td>
<td>11.60</td>
<td>D, H</td>
<td>D</td>
</tr>
<tr>
<td>Northern Sweden</td>
<td>1969-1980</td>
<td>6</td>
<td>128</td>
<td>4.70</td>
<td>E, H, M</td>
<td>A, D, O</td>
</tr>
<tr>
<td>Cantabria, Spain</td>
<td>1974-1985</td>
<td>3</td>
<td>65</td>
<td>4.60</td>
<td>E</td>
<td>D, H, I</td>
</tr>
<tr>
<td>Sardinia, Italy</td>
<td>1957-1980</td>
<td>4</td>
<td>182</td>
<td>2.20</td>
<td>F</td>
<td>D</td>
</tr>
<tr>
<td>South-West Greece</td>
<td>1990-2003</td>
<td>2</td>
<td>133</td>
<td>1.50</td>
<td>E</td>
<td>Bi, F, I, N</td>
</tr>
<tr>
<td>Belgrade, Yugoslavia</td>
<td>1985-1991</td>
<td>1</td>
<td>58</td>
<td>1.70</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1989-1993</td>
<td>1</td>
<td>84</td>
<td>1.20</td>
<td>E, C, G, H</td>
<td>Bi, F, H, J, L</td>
</tr>
<tr>
<td>Northern Denmark</td>
<td>1974-1986</td>
<td>5</td>
<td>186</td>
<td>2.70</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td><strong>Group 3: prospective case series</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas, USA</td>
<td>1982-1994</td>
<td>114</td>
<td>1200</td>
<td>9.50</td>
<td>E</td>
<td>E, N</td>
</tr>
<tr>
<td>New York, USA</td>
<td>1972-1977</td>
<td>33</td>
<td>668</td>
<td>4.90</td>
<td>M</td>
<td>D</td>
</tr>
<tr>
<td>Colorado, USA</td>
<td>1989-1991</td>
<td>4</td>
<td>167</td>
<td>2.40</td>
<td>C</td>
<td>D, E, N</td>
</tr>
<tr>
<td><strong>Group 4: retrospective case series</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>1977-2004</td>
<td>7</td>
<td>251</td>
<td>2.80</td>
<td>E</td>
<td>Bi, G, I, K, N</td>
</tr>
<tr>
<td>London, UK</td>
<td>1965-1984</td>
<td>27</td>
<td>580</td>
<td>4.70</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Rochester, USA</td>
<td>Pre 1955</td>
<td>5</td>
<td>58</td>
<td>9</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>Laus, Italy</td>
<td>1987-2007</td>
<td>23</td>
<td>531</td>
<td>4.30</td>
<td>E</td>
<td>Bi, N</td>
</tr>
<tr>
<td>Reina Sc Cons, Canada</td>
<td>Pre 1974</td>
<td>3</td>
<td>57</td>
<td>5.80</td>
<td>E</td>
<td>D, F, H, J, I</td>
</tr>
<tr>
<td>Leuven, Belgium</td>
<td>1991-1995</td>
<td>12</td>
<td>140</td>
<td>8.80</td>
<td>E</td>
<td>Bi, H, J, L</td>
</tr>
<tr>
<td>Israel</td>
<td>1959-1973</td>
<td>3</td>
<td>318</td>
<td>1</td>
<td>C, I, M</td>
<td>C, D, E, N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Key</th>
<th>Ascertainment</th>
<th>Key</th>
<th>Diagnostic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Prospective registry</td>
<td>A</td>
<td>Post mortem</td>
</tr>
<tr>
<td>B</td>
<td>All neurologists</td>
<td>B</td>
<td>El Escorial criteria</td>
</tr>
<tr>
<td>C</td>
<td>Multicentre hospital records</td>
<td>Bi</td>
<td>EEC definite, probable</td>
</tr>
<tr>
<td>D</td>
<td>Multicentre discharge records</td>
<td>Bi</td>
<td>EEC definite, probable, suspect</td>
</tr>
<tr>
<td>E</td>
<td>Single centre hospital records</td>
<td>C</td>
<td>Simplified EFN criteria</td>
</tr>
<tr>
<td>F</td>
<td>Single centre discharge records</td>
<td>D</td>
<td>Diagnosed by neurologist pre-criteria</td>
</tr>
<tr>
<td>G</td>
<td>ALS association records</td>
<td>E</td>
<td>ALS rated using institutions own scoring system</td>
</tr>
<tr>
<td>H</td>
<td>Death certificates</td>
<td>F</td>
<td>SMA included</td>
</tr>
<tr>
<td>I</td>
<td>Compulsory reporting</td>
<td>G</td>
<td>SMA excluded</td>
</tr>
<tr>
<td>J</td>
<td>EMG archives</td>
<td>H</td>
<td>PMA included</td>
</tr>
<tr>
<td>K</td>
<td>Control drug registry</td>
<td>I</td>
<td>PMA excluded</td>
</tr>
<tr>
<td>L</td>
<td>Private neurologists</td>
<td>J</td>
<td>PLS included</td>
</tr>
<tr>
<td>M</td>
<td>Questionnaire</td>
<td>K</td>
<td>PLS excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>PSP included</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>PSP excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>EMS &gt;50% patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>O</td>
<td>EMS &lt;50% patients</td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; EEC, El Escorial criteria; FALS, familial amyotrophic lateral sclerosis; PMA, progressive muscular atrophy; PSP, progressive supranuclear palsy; SMA, spinal muscular atrophy; WFN, World Federation of Neurology.
Statistical analysis
In each study the rate of FALS is reported as the number of familial cases among all cases of ALS. Statistical analysis was carried out to combine proportions using the Meta function of R (R Development Core Team 2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 http://www.R-project.org/.

The inverse variance method was used to pool proportions. Both fixed and random effects models were estimated and 95% CIs were calculated.

RESULTS
Systematic review
Fifty-four epidemiological studies provided original information on cohorts of patients with ALS. A rate of FALS was reported in 38 of these studies, although in no case was FALS ascertainment the primary objective. Five studies were not included in the analysis. Of these, two were undertaken to genotype mutations in the ALS population. Both studies exhibited enrolment bias. Three studies reported data on more than one occasion and therefore only the most recent study was included in the meta-analysis. Reports from geographical areas with high incidence clusters (Guam/Ri Peninsular of Japan) were excluded.

Of the 33 studies analysed, eight reported incidence data from prospective population based registers; 14 reported retrospective incidence and prevalence data; three reported data from prospective case series of disease progression, and eight reported data from retrospective case series. Only two studies stated how they defined FALS. In total, 575 cases of FALS were identified among 11,221 reported cases of ALS.

The type of study, rate of FALS, source of case ascertainment and case inclusion criteria are outlined in Table 1.

Meta-analysis
Pooled analysis of all studies generated a rate of FALS of 4.6% (95% CI 3.9% to 5.5%), (table 2).

A degree of heterogeneity was noted between reported rates of FALS in the prospective population based registers available for analysis. The pooled result for rate of FALS for these studies was 5.1% (95% CI 4.1% to 6.1%). In the 14 retrospective population based studies, the pooled result for rate of FALS was 3.7% (95% CI 2.9% to 4.7%).

Figure 1 demonstrates a forest plot of the results. The individual rate of FALS for each country in Europe was plotted on a map to determine a possible geographic pattern (figure 2).

Table 2 Meta-analysis results: subgroup analysis and pooled analysis

<table>
<thead>
<tr>
<th>Study type</th>
<th>Proportion FALS cases (95% CI)</th>
<th>Total ALS cases</th>
<th>Fixed effects</th>
<th>Random effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1: prospective population based registry</td>
<td>6</td>
<td>205</td>
<td>5263</td>
<td>5.0% (4.4 to 5.6)</td>
</tr>
<tr>
<td>Group 2: retrospective population based study</td>
<td>14</td>
<td>59</td>
<td>1666</td>
<td>3.7% (2.9 to 4.7)</td>
</tr>
<tr>
<td>Group 3: prospective case series</td>
<td>3</td>
<td>151</td>
<td>2035</td>
<td>7.3% (6.2 to 8.4)</td>
</tr>
<tr>
<td>Group 4: prospective case series</td>
<td>8</td>
<td>100</td>
<td>2237</td>
<td>4.4% (3.8 to 5.2)</td>
</tr>
<tr>
<td>Population based studies (groups 1 and 2)</td>
<td>27</td>
<td>324</td>
<td>6949</td>
<td>4.3% (4.2 to 5.3)</td>
</tr>
<tr>
<td>Case series studies (groups 3 and 4)</td>
<td>11</td>
<td>251</td>
<td>4272</td>
<td>7.5% (5.0 to 6.4)</td>
</tr>
<tr>
<td>Pooled results for all studies (groups 1–4)</td>
<td>32</td>
<td>575</td>
<td>11,221</td>
<td>5.1% (4.7 to 5.5)</td>
</tr>
</tbody>
</table>

FALS, familial amyotrophic lateral sclerosis.


DISCUSSION
Our analysis indicates that the commonly accepted frequency of 10% for FALS represents an overestimation of the dominantly inherited Mendelian form of ALS. The population based frequency of 5.1% is likely to represent the most accurate estimation of rate of FALS, as population based studies include all patients in a defined geographic area in a given period of time. Accordingly, reported rates of FALS of 10% from previous studies are likely to have been biased by ascertainment from populations enriched by FALS cases.

Geographic variation in the rate of FALS in Europe (figure 2) may reflect true population based differences across different regions. Heterogeneity in the genetic substructure of European populations has been demonstrated recently. These geographic differences may occur because of variation in the underlying genetic structure of the European population. However, difficulties in case ascertainment could also account for this difference. Evidence for population based differences is emerging for SOD1: A founder effect for the A4V mutations in SOD1 has been recently identified in the USA and this mutation is rare in Europe. Mutations in C9orf72 seem to be primarily in Japanese populations. The existence of geographic variability for TARDP and FUS has yet not been established. As more causative genes are identified in FALS, detailed analysis of the frequency of various mutations within individual FALS populations will become available.

The meta-analysis is limited by the absence of a clear definition of FALS. While most groups define FALS as the presence of ALS in at least two members of an extended kindred, the degree of relatedness and the size of the extended kindred are rarely considered. Given that the lifetime risk of ALS is 1:450 for women and 1:350 for men, there is an increasing probability of two affected members with sporadic ALS occurring in the same kindred as the size of the kindred increases. Therefore, ensuring segregation of mutations within kindreds is also
important, as exemplified by a recent report of two apparent SOD1 kindreds in which the disease did not segregate with a known pathogenic SOD1 mutation.\(^\text{11}\)

In conclusion, we have demonstrated that the rate of FALS across population-based studies rarely exceeds 5% of all cases of ALS. This contrasts with the generally accepted figure of 10%, which originated from a paper written in 1955.\(^\text{12}\)

Careful prospective population-based analysis of kindreds using validated criteria for a diagnosis of FALS is required to confirm that 5% represents the true population-based rate of familial ALS, and to determine whether the documented geographic variation in rates of FALS is corroborated.

Funding This work was supported by the INVIDA, IMNDRF and HRB funding.

Competing interests OH is a HRB Clinician Scientist. Her group has received unrestricted research grants from Merck Serono, Biogen Idc and Bayer Schering. She has received honoraria for providing expert advice to Merck Serono, Biogen Idc, Janssen Cilag, Allergan, Oto Pharmaceuticals and Cyther.

Provenance and peer review Not commissioned, externally peer reviewed.

REFERENCES

SHORT REPORT

Proposed criteria for familial amyotrophic lateral sclerosis

SUSAN BYRNE1,2, PETER BEDE1,2, MARWA ELAMIN1,2, KEVIN KENNA1, CATHERINE LYNCH2, RUSSELL MCLAUGHLIN3 & ORLA HARDIMAN1,2

1TCIN, Trinity College, Dublin, Ireland and 2Department of Neurology, Beaumont Hospital, Dublin, Ireland

There is currently no consensus on the definition of familial ALS (FALS). We propose criteria for FALS that incorporate family history and genetic analysis. The aim is to increase the yield of genes causing FALS and to facilitate comparative interpretation of epidemiological and genetic FALS data.

Familial amyotrophic lateral sclerosis (FALS) is clinically indistinguishable from sporadic amyotrophic lateral sclerosis (ALS) and accounts for 5% of reported cases of ALS (1). ALS is generally accepted to be familial if one or more first- or second-degree relatives are reported to suffer from the condition (2). However, lack of a clear definition leads to difficulty in interpreting epidemiological and genetic studies.

A recent review of all epidemiological studies reporting rates of FALS showed that only 6% (2/33) of studies provided a definition for FALS. Similarly, only two of 13 (15%) papers reporting the rate of SOD1 mutations in specific cohorts define the term FALS, and the two definitions differ (3,4).

Accurate reporting of the rate of FALS within a cohort is dependent on the availability of a detailed family history from every patient diagnosed with ALS. A number of factors can confound the ascertainment of familial disease (5) (Table I).

It has been recently reported that kindreds comprising only two affected family members account for up to 50% of all FALS cases (2). Using both information for lifetime adjusted individual risk of dying from ALS (1:350 males and 1:472 females) (6,7) and kindred size, it is possible to calculate the probability of a second person within a kindred developing ALS by chance. It is apparent from Figure 1 that two cases of sporadic ALS within a large kindred could account for a sizeable proportion of currently reported FALS kindreds. Kindreds with three or more reported cases of ALS are less likely to be attributable to chance (Figure 1) and more likely to be due to a direct genetic effect.

Mutations in SOD1 are reported to account for up to 20% of FALS, although reported rates of SOD1 in different cohorts vary widely. While it is acknowledged that there may be geographic and population based variation in the prevalence of SOD1 mutations, this may not fully explain the disparity seen between geographically neighbouring cohorts (3,9). Although studies that have drawn from larger kindreds with more affected members may expect to record higher rates of SOD1 mutations, proof of segregation of disease with the pathogenic mutation is essential. This has been evidenced by recent reports of SOD1 kindreds in which mutations were absent in some affected members (10), suggesting that in some cases sporadic ALS is misclassified as familial within FALS kindreds. (These assumptions are based on the premise that heritability is due to genetic effect rather than an environmental factor to which family members are exposed.) To clarify matters, we propose that FALS be categorized in to three groups: definite, probable and possible FALS (Table II).

We propose that kindreds with three or more affected members, and kindreds with two or more affected members with gene positive cosegregation, should be categorized as ‘definite FALS’.

We propose that kindreds with one affected first- or second-degree relative should be categorized as ‘probable FALS’. For classification of possible FALS we suggest three scenarios: 1) the presence of ALS in a distant relative; or 2) a documented mutation in a known FALS gene in a patient with apparently sporadic ALS; or 3) the presence of frontotemporal dementia (FTD) in a first-degree relative. FTD is known to occur in a subset of patients with ALS, and some kindreds with known mutations have been shown to comprise members with both pure ALS

Correspondence: Susan Byrne, Beaumont Hospital, Dublin 9, Ireland. E-mail: susanbyrne@gmail.com.

303
False negative reporting due to:
- lack of information on older generations
- the patient being the first in their family to develop the disease and not informing the centre when a subsequent relative is diagnosed
- misdiagnosis of a relative with another condition
- relatives dying earlier of other causes
- low gene penetrance and small family size causing the disease to have the appearance of sporadic disease
- denial

False positive reporting due to:
- patients overcalling the diagnosis of ALS in relatives
- over-reporting of ALS on death certificates

and pure FTD (11,12). Therefore, in cases where one family member is affected with ALS but where there appears to be aggregation of FTD in other family members, we propose that the condition should be considered as 'possible FALS'. The presence of expanded phenotypes should be categorized separately pending the outcome of detailed prospective family aggregation studies.

At present it is not possible to test this classification system using published FALS kindreds because few studies have provided details regarding kindred size and number of affected family members. To achieve this, prospective validation of the proposed criteria for FALS will be required, and the new classification system should be tested using familial cases of ALS from population based cohorts. This will facilitate the comparison of rates of FALS across different geographic and ethnic populations, and will permit an accurate comparison of reported rates of known genes including SOD1, FUS, ANG, TARDP, Optineurin and others.

Table II. Proposed criteria for familial amyotrophic lateral sclerosis.

| Definite FALS | ALS patient with at least two first- or second-degree relatives with ALS |
| Probable FALS | ALS patient with at least one relative with ALS and gene-positive cosegregation |
| Possible FALS | ALS patient with one first- or second-degree relative with ALS |

Note: For all families record the size of the kindred and the relationship between the affected individuals.
- First-degree relatives: parents, children and siblings.
- Second-degree relatives: grandparents, aunts/uncles.

Such a categorization of FALS will ultimately standardize epidemiological and genetic studies, and will allow better predictive models for genetic counselling purposes.

In the meantime, we propose that a consensus definition of FALS should be adopted, and that these criteria should be considered as part of the revised El Escorial diagnostic criteria for ALS (13,14).

Acknowledgements
This work was supported by the Irish Motor Neuron Disease Association (IMNDA), Irish Motor Neuron Disease Association (IMNDA).
Proposed criteria for FALS

Disease Research Foundation (IMNDRF) and Health Research Board (HRB) funding. Orla Hardiman is an HRB Clinician Scientist. Her group has received unrestricted research grants from Merck Serono, Biogen Idec and Bayer Schering. She has received honoraria for providing expert advice to Merck Serono, Biogen Idec, Janssen Cilag, Allergan, Ono Pharmaceuticals and CyRx.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References
SHORT REPORT

Absence of consensus in diagnostic criteria for familial neurodegenerative diseases

Susan Byrne,1,2 Marwa Elamin,1,2 Peter Bede,1,2 Orla Hardiman1,2

ABSTRACT

Background A small proportion of cases seen in neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS), Parkinson’s disease and Alzheimer disease are familial. These familial cases are usually clinically indistinguishable from sporadic cases. Identifying familial cases is important both in terms of clinical guidance for family members and for gene discovery.

Method Surveys assessing the definition of familial amyotrophic lateral sclerosis (FALS) were completed by clinicians with an interest in ALS.

Results 95 surveys were completed by respondents from 15 countries. A third of total respondents stated that they thought that neurologists were using the same definition for FALS (33.3%, 30). No consensus was achieved among clinicians when provided with five different definitions for FALS. However, the preferred definition was ‘a patient with ALS with either a first or second degree relative also suffering from ALS’ (37.8%, 31).

Conclusion There is no consensus on a standard definition for FALS among clinicians. It is likely that similar inconsistencies apply to other conditions, such as Parkinson’s disease and Alzheimer disease, in which both familial and sporadic diseases occur. Inconsistent classification could hinder gene discovery.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting 1 in 400 people in their lifetime.1 A recent meta-analysis reported that the rate of familial amyotrophic lateral sclerosis (FALS) was 5%2. However, only 6% (2/33) of studies included in the meta-analysis provided a definition for FALS. Similarly, only 2 of 13 (15%) of papers reporting the rate of SOD1 mutations in specific cohorts define the term FALS, and the two definitions differ.3 These findings pose the question as to the precise definition of FALS, and as to the presence of a uniformly applied classification among clinicians.

We designed and administered an online questionnaire, which was distributed to clinicians involved in the diagnosis and management of ALS. The survey had three aims: to determine whether a consensus exists among clinicians regarding a standard definition for FALS, to examine clinical practice in the diagnosis of FALS and to seek opinion as to the need for a consensus meeting on the definition of FALS.

METHOD

Based on a series of qualitative interviews with key opinion leaders in ALS, a 26-item questionnaire was devised (supplementary appendix e-1). The study was classified and approved as an audit by the local IRB. The questionnaire sought information in six domains:

► demographic information (A)
► is there a standard definition for FALS in use by neurologists? (B)
► the definition for FALS used by the respondent in their clinical practice (C)
► existing practice among responding clinicians when confronted with a possible FALS pedigree (D)
► desire for a consensus meeting to define FALS (E)

The questionnaire was distributed using Survey-Monkey® to ALS mailing lists in Europe (ENCALS), North America (NEALS), Australia and India.

RESULTS

Demographic information (A)

There were 95 respondents from 15 countries (see supplementary table 1). In all, 61 (64.9%) of the respondents were male subjects. Sixty-eight (75.6%) of the respondents were neurologists with the remainder being trainee neurologists and clinical geneticists (21.1% and 4.4%, respectively). Eighty (85.1%) declared that they had a special interest in ALS.

Is there a standard definition for FALS in use among neurologists? (B)

Respondents were asked if they thought that there was a standard definition among neurologists for FALS. A third of total respondents stated that neurologists were using the same definition for FALS (33.3%, 30). There was a statistically significant difference when subgroup analysis based on country of practice was carried out: over half of respondents from North America (51.4%, 18) stated that there was a standard definition for FALS in use among neurologists in comparison with less than a quarter of respondents from Europe (22.0%, 9) (p=0.015).

To explore the definition for FALS used by the respondent in their clinical practice (C)

Respondents were provided with five possible definitions for FALS. They were asked to select the definition that in their opinion is most applied in publications relating to FALS. There was no consensus among the respondents (see Q2 results, table 1).
Table 1: Definition for FALS—a patient with ALS who has

<table>
<thead>
<tr>
<th>Option</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>One 1st degree relative with ALS</td>
</tr>
<tr>
<td>ii)</td>
<td>Two or more 1st degree relatives with ALS</td>
</tr>
<tr>
<td>iii)</td>
<td>Either one 1st OR 2nd degree relative with ALS</td>
</tr>
<tr>
<td>iv)</td>
<td>Any relative with ALS, no matter how distant</td>
</tr>
<tr>
<td>v)</td>
<td>A positive gene test for a known ALS gene, but no family history</td>
</tr>
</tbody>
</table>

Respondents were then provided with the same five definitions and asked to select the one that most matched their own clinical practice (see Q2 results, table 1). Again, there was no consensus, but the preferred definition was 'a patient with ALS with either a first or second degree relative also with ALS' (37.8%, 31).

In subgroup analysis, the preferred response for the definition of FALS among respondents who stated that they had a special interest in genetics and FALS was 'a patient with ALS with any relative with ALS, no matter how distant' (34.8%, 8).

If the respondents did not use any of the given definitions in their clinical practice they were provided with an option to add their own definition.

To evaluate practice among responding clinicians when given a kindred scenario (D) Respondents were then provided with eight pedigrees and asked whether they would diagnose the proband with FALS (figure 1).

As in (A) and (B), the level of consensus was high. However, in over half of the pedigrees the majority of respondents chose the 'Maybe' option. In all, 51 (38.6%) respondents gave answers that differed from the definition that they had previously stated that they used in clinical practice.

Fifty-nine (67.0%) of respondents stated that they carried out genetic testing on patients who had a family history of ALS. Only 9 (10.8%) respondents stated that they routinely carry out genetic testing on ALS patients with no family history.

In all, 52.3% (45) of respondents stated that they refer patients with FALS for genetic counselling.

To assess the need for a consensus meeting among clinicians (E) Sixty-five (74.7%) respondents agreed that a consensus meeting would be helpful in defining FALS.

DISCUSSION

This study demonstrates that there is no standard definition for FALS. Although the majority of respondents agreed that having one other affected family member with ALS was sufficient to constitute a diagnosis of FALS, opinions differed as to whether this relative should be at the very least a first-degree relative, a second-degree relative or any relative, no matter how distant (34.8%, 8).

Notwithstanding, over a third of respondents believe that the term FALS carries a standard definition among specialists. Despite this, in a series of eight kindred scenarios the majority of respondents chose the 'maybe' option in four of the scenarios, indicating a lack of certainty as to what constitutes the familial...
form of the disease. The greatest degree of uncertainty was observed in scenarios where a distant relative was affected. Moreover, even when clinicians had clearly stated their preferred definition of FALS, over half of respondents were inconsistent in their definition when confronted with specific scenarios.

As is the case for all conditions in which there is both a familial and sporadic form, a clear and uniformly applied definition is important. We have shown previously that there are large differences in the reported rates of FALS across different countries. While it is plausible that these differences are due to a founder effect with any given population, it is also conceivable that these differences arise from differing definitions for the term FALS. The same is true for reported rates of SOD1, which vary widely among different research cohorts. Again, this disparity may be due to a true difference in the rate of SOD1. However, it is also possible that these differences are a function of inconsistencies in classification.

A consensus definition of what is familial disease is also important when selecting kindreds for linkage and exome sequencing. Among respondents with an interest in ALS genetics and familial ALS, the most common definition for FALS was 'any relative with ALS, no matter how distant.' We have shown previously that chance plays a role in a high proportion of ALS kindreds where only two members are affected with ALS and that true role of genetics in a kindred where only two members are affected may be considerably lower than that where three or more members are affected. Therefore, research groups that study high proportions of kindreds with only two affected members are likely to observe a rate of SOD1 mutations that is lower than groups that draw from kindreds with larger affected numbers (see supplementary figure 1). A recent paper by Al-Chalabi and Lewis demonstrated that family size and gene penetrance directly contribute to observed inheritance patterns within a kindred, and that poorly penetrant genes in small families may appear to cause sporadic disease.

A further factor to consider is that poorly ascertained family histories may mask familial disease. It is important to take a thorough family history from a reliable source. The clinician must acknowledge the many reasons that a patient may not report a family history of ALS or frontotemporal dementia (FTD) when in fact one does exist, including lack of information about past generations, misdiagnosis, relatives dying of another condition before they develop ALS and denial. Moreover, the recent identification of an expanded hexanucleotide repeat in families with both ALS and FTD further underlines the importance of obtaining an extensive family history as possible in apparently sporadic cases, and neurologists in training should be educated to this effect. It should also be noted that familial disease does not always imply a genetic origin, although in the case of ALS, no definitive environmental exposure has been identified to date.

In all, 75% of respondents agreed that there should be a consensus meeting on the definition of FALS. Such a meeting should include both neurologists and geneticists. Outcomes from such a consensus should be an agreed definition that is clear, concise and clinically applicable. An additional research requirement could be that clinicians record the total number of people within the kindred and the degree of relatedness of the other affected family member. Certainty as to the definition of FALS will render epidemiological and genetic studies more transparent and will in turn permit more accurate comparison between geographic regions.

CONCLUSION
It is likely that similar inconsistencies apply to other conditions, such as Parkinson's disease and Alzheimer's disease, in which both familial and sporadic diseases occur. A consensus initiative to provide precise definitions of familial and sporadic disease is urgently required.

Contributors SB: designed the questionnaire, administered the questionnaire, carried out the statistical analysis and wrote the manuscript. ME provided advice on questionnaire design and content and was involved in manuscript review. PB provided advice on questionnaire design and content and was involved in manuscript review. OH provided advice on questionnaire design and content. OH advised on manuscript content and edited all versions of the manuscript.

Funding This work was funded by the Health Research Board, Ireland. Prof Hardiman is an HRB Clinician Scientist. Her group has received unrestricted research grants from Merck Serono, Biogen Idec and Bayvie Schoening. She has received honoraria for providing expert advice to Merck Serons, Biogen Idec, Janssen Cilag, Allergan, Ono Pharmaceuticals and Cybra.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Statistical and data tables are available from the corresponding author (swallyrne@gmail.com).

REFERENCES
Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study

Susan Byrne, Marwa Elamin, Peter Bede, Abdulaziz Statonou, Cathal Walsh, Berrie Cor, Mark Heverin, Norah Jordan, Kevin Kennedy, Catherine Lynch, Russell Laloue, Padraig Mahadeva Iyer, Caoimhe O'Brien, Jude Phukan, Bronna Wynne, Aron L. Bode, Daniel G Bradley, Nad Pender, Amin Al-Chalabi, Orla Hardiman

Summary

Background Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of upper and lower motor neurons, associated with frontotemporal dementia (FTD) in about 14% of incident cases. We assessed the frequency of the recently identified C9orf72 repeat expansion in familial and apparently sporadic cases of ALS and characterised the cognitive and clinical phenotype of patients with this expansion.

Methods A population-based register of patients with ALS has been in operation in Ireland since 1995, and an associated DNA bank has been in place since 1999. 435 representative DNA samples from the bank were screened using repeat-primed PCR for the presence of a GGGGCC repeat expansion in C9orf72. We assessed clinical, cognitive, behavioural, MRI, and survival data from 191 (44%) of these patients, who comprised a population-based incident group and had previously participated in a longitudinal study of cognitive and behavioural changes in ALS.

Findings Samples from the DNA bank included 49 cases of known familial ALS and 386 apparently sporadic cases. Of these samples, 20 (41%) cases of familial ALS and 19 (5%) cases of apparently sporadic ALS had the C9orf72 repeat expansion. Of the 191 patients for whom phenotype data were available, 21 (11%) had the repeat expansion. Age at disease onset was lower in patients with the repeat expansion (mean 56.3 [SD 8.3] years) than in those without (61.3 [10.6] years; p=0.043). A family history of ALS or FTD was present in 18 (86%) of those with the repeat expansion. Patients with the repeat expansion had significantly more co-morbid FTD than patients without the repeat (50% vs 12%), and a distinct pattern of non-motor cortex changes on high-resolution 3 T magnetic resonance structural neuroimaging. Age-matched univariate analysis showed shorter survival (20 months vs 26 months) in patients with the repeat expansion. Multivariable analysis showed an increased hazard rate of 1.9 (95% 1.1-3.7; p=0.035) in those patients with the repeat expansion compared with patients without the expansion.

Interpretation Patients with ALS and the C9orf72 repeat expansion seem to present a recognisable phenotype characterised by earlier disease onset, the presence of cognitive and behavioural impairment, specific neuroimaging changes, a family history of neurodegeneration with autosomal dominant inheritance, and reduced survival. Recognition of patients with ALS who carry an expanded repeat is likely to be important in the context of appropriate disease management, stratification in clinical trials, and in recognition of other related phenotypes in family members.


Introduction Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of upper and lower motor neurons. Cognitive impairment occurs in up to 50% of cases, and one in seven patients develops frontotemporal dementia (FTD).1 The existence of families with pure ALS. pure FTD, and ALS with co-morbid FTD (ALS-FTD) has been long recognised.1 A combination of clinical, neuroimaging, and neuropathological data suggest that ALS and FTD might form part of a disease continuum, with pure ALS at one extreme and pure FTD at the other. Detailed genetic studies including conventional linkage2 and genome-wide association studies of families with ALS and FTD have identified a reproducible locus on chromosome 9p21.3,4 and a disease-segregating expanded hexanucleotide repeat in the C9orf72 gene in that locus accounts for up to 60% of familial ALS and up to 10% of sporadic ALS.5 Preliminary data suggest that hexanucleotide expansions of more than 23 are pathological, although further population-based control studies are warranted.6 Detailed phenotyping of patients with this pathological expansion has yet to be reported.

In this study, we characterised the clinical features, demographics, survival, neurocognitive profile, family history, and neuroimaging findings in a population-based cohort of Irish patients carrying the C9orf72 hexanucleotide repeat expansion.
Methods

Participants and study design

A population-based register of patients with ALS has been in operation in Ireland since 1995," and an associated bank of DNA extracted from venous leucocytes has been in place since 1999. 435 representative samples were selected for screening from the DNA bank on the basis of the following criteria: Irish origin; both incident and prevalent cases; sufficiently high-quality and quantity to permit subsequent Southern blotting; and proportionate representation of the familial and sporadic ALS contained in the DNA bank. 188 age-matched, sex-matched, and geographically matched controls were specifically selected through the patients’ primary care provider for this study.

Of these 435 samples, 191 belonged to population-based incident patients diagnosed with ALS from November, 2006, to May, 2011, who were selected through the ALS register and enrolled in a prospective longitudinal case-control study of cognitive and behavioural function (webappendix p 2)." Detailed longitudinal clinical, neurocognitive, and behavioural data, structural MRI, and survival data have been gathered on this cohort, and DNA has been banked for genomic analysis. Patient enrolment to the ALS register was achieved by direct referral by all neurologists and neurophysiologists practising in Ireland and by close and regular interaction with community-based primary-care and disability services. For inclusion in the ALS register and enrolled in a prospective longitudinal case-control study of cognitive and behavioural function, participants had to fulfill standard neurological diagnostic criteria for ALS, be free of co-existing neurological conditions, and have a confirmed diagnosis of ALS by a neurologist. For the present study, DNA samples from 191 incident and prevalent cases were selected for an associated case-control study of cognitive and behavioural function. The exclusion criteria for the prospective longitudinal case-control study included a history of traumatic brain injury, learning disability, alcohol dependence, type 1 or uncontrolled type 2 diabetes mellitus, and serious active mental illness. A neuropsychological battery was used to assess cognitive domains, including executive function (Stroop, Brixton, Verbal Fluency, Category Fluency, Backwards Digit Span), memory function (Logical Memory, Rey Osterreith Figure Test Immediate, and Delayed, Visual Paired Associates, California Verbal Learning Test), language (Boston Naming Test), visuospatial domains (Rey Osterreith Figure Test Copy), and behaviour (Frontal Systems Behavior Scale)." Matched controls were recruited for comparative purposes. Controls for the prospective longitudinal case-control study were recruited through a network of volunteers obtained via advertisement and through the patients’ primary care providers. Details of the methods and categorisation have been described previously." Patients completed a validated family history questionnaire,*^ and were subsequently contacted by relatives with clinical features suggestive of familial ALS. All reported deaths within the cohort were validated using ONS death certification and posthumous classification was made on the basis of all clinical details available including age of onset, rate of disease progression, and type of dementia. A family history of FTD was defined when a first-degree or second-degree relative had any of the following criteria: a clinical diagnosis of FTD; and through the care of a neurologist or a clinician, confirmed by review of clinical notes: a clinical diagnosis of Pick’s disease confirmed by death certification; and documentation of onset of dementia before the age of 65 years and with striking personality change suggestive of clinical features characteristic of Alzheimer’s disease or multi-infarct dementia were not
recorded as having FTD. Family history was classed as strong when three or more first-degree or second-degree relatives were affected in each pedigree.

3 T magnetic resonance grey-matter voxel-based morphometry analysis was done in ten patients with the repeat expansion and 30 without (webappendix p 1). The demographic details (age, disease duration, and clinical phenotype) of the two groups were matched. A study-specific template was created, to which the grey-matter images from each patient were non-linearly co-registered. A voxel-wise generalised linear model was used to test for differences between the groups using permutation-based non-parametric testing. A minimum cluster size of 800 μL was applied to the results of the analysis to show only significant regions of differences. Significance was set at p<0.05 (voxel level) and corrected for multiple comparisons at p<0.05 (family-wise error)."
Articles

expansion. In total, 18 of 21 patients with the repeat behaviour in ALS. Analysis of sequence data generated in the longitudinal case-control study of cognition and 435 samples from the DNA bank, comprising the 191 patients in this cohort carried the potentially new pathogenic variants. 21 (11%) of the population-based incident group recruited to participate carried a truncated 18-SNP version containing the T allele by Mok and colleagues, whereas the remaining three carried a truncated 18-SNP version containing the T allele for rs822723 at the telomeric end of the 20-SNP haplotype.

Phenotype data were available for 191 (44%) of the 435 samples from the DNA bank, comprising the population-based incident group recruited to participate in the longitudinal case-control study of cognition and behaviour in ALS. Analysis of sequence data generated for SOD1, TARDBP, and FUS showed no known or potentially new pathogenic variants. 21 (11%) of the 191 patients in this cohort carried the C9orf72 repeat expansion. In total, 18 of 21 patients with the repeat expansion had a verified first-degree or second-degree relative with either ALS (12 patients) or FTD (six patients). Of those who did not carry the C9orf72 repeat (7D patients) only three (3%) described a family history of FTD (p=0.0001).

Of the three remaining patients carrying an expanded repeat, who apparently had sporadic ALS, one had a strong family history of depressive illness with suicide, one had a family history of parkinsonism in two paternal siblings and unspecified dementia and mobility problems in a paternal grandmother, and one was unable to provide a family history because both parents had died prematurely. Construction of multiple generation pedigrees was possible in kindreds from 15 of 21 patients with the repeat expansion. A dominant inheritance pattern was apparent in all cases, although six of 15 exhibited a pattern of apparently incomplete penetrance in which obligate carriers survived into the eighth or ninth decade of life.

Age at disease onset and diagnosis was lower in patients with the repeat expansion than in those without, and time from symptom onset to diagnosis did not differ between those with the repeat expansion and those without (14-7 months for carriers vs 13-2 months for non-carriers; p=0.541; table 1). Sex, site of disease onset, and ALS functional rating score did not differ at first assessment between the two groups. All 191 patients in the population-based incident cohort had previously undergone detailed neuropsychological testing and 186 (97%) had been classified into one of four cognitive categories: ALS-FTD, executive impairment, non-executive impairment, and normal cognition. Five (3%) of 191 patients were too unwell to complete the full cognitive battery and were therefore not classified according to cognitive criteria. However, behavioural data were available from all five, and these data were included in the analysis. Of the 186 patients who completed full cognitive testing at the time of first assessment, 91 (49%) underwent further testing after 6 months and 34 (18%) had a third assessment 1 year after initial assessment. Detailed behavioural data from the Frontal Systems Behavioural Scale were available for 130 (68%) of 191 patients. C9orf72 repeat expansion carriers exhibited a characteristic profile, with a significantly higher frequency of co-morbid FTD (50% vs 12%) in the group with the expanded repeat (table 2). In a univariate ANCOVA analysis comparing performance on executive tasks (category fluency, verbal fluency index, Brixton spatial anticipation task, Stroop interference task, and backward digit span tests) between patients with the repeat expansion and those without, after adjustment for differences in age at time of assessment, patients with the repeat expansion had significantly more impairment according to the Brixton scaled score than did those without the repeat (table 3).

Further analysis of the subgroup of patients with ALS-FTD (30 patients) showed that a third of patients carried
the repeat expansion (table 4). Patients with ALS-FTD and a repeat expansion were significantly younger when symptoms of ALS appeared. Other differences noted in patients with ALS-FTD carrying the repeat expansion included increased rate of spinal onset, predominance of behavioural variant FTD, and younger age at diagnosis, although these differences were not significant (table 4). The mean language score of patients with the C9orf72 repeat on the Boston naming test was higher (20.1 [IQR 5.5-35.5]) in carriers vs 13.5 [8-9] in non-carriers; p=0.043).

Of the patients who had been classified as having normal cognition in our previous report, patients with the repeat expansion exhibited a higher rate of behavioural impairment than did patients without the repeat expansion (four of six vs 11 of 50; p=0.038). Patients with the repeat expansion had significantly higher apathy scores (18.0 [IQR 12.5-35.5]; p=0.032) and dysexecutive behaviour (8.0 [IQR 2.5-27.0]; p=0.023; webappendix p 1).

Of the 21 patients with the repeat expansion, only two were classified as having neither cognitive impairment nor behavioural impairment at the time of their first assessment. Follow-up testing was not available for either patient; one patient had a strong family history of ALS and the other had a strong family history of FTD. None of the 63 patients who had previously been classed as behaviourally normal and had a negative family history had the repeat expansion.

Compared with patients without the repeat expansion (82 patients), those with the expansion (nine) had a significantly faster rate of motor progression according to total ALS functional rating scale (revised) scores (median decline of 1.54 [IQR 0.07-2.08] points per month in carriers vs 0.83 [IQR 0.34-4.13] points per month in non-carriers; p=0.009). The spinal subscore of the ALS functional rating scale showed that this difference was driven by a higher rate of decline in spinal function (median decline of 1.2 [IQR 0.77-1.53] points in carriers vs 0.5 [IQR 0.17-0.83] points per month in non-carriers; p=0.016).

Age-matched univariate analysis showed significantly shorter survival in patients with the repeat expansion (21 patients; median survival 20 months, 95% CI 8.9-31.1) than in patients without the expansion (570.26 months, 21-1.30-9; p=0.017; figure 1). Presence of the repeat expansion generated a significant hazard ratio (1.9, 95% CI 1.1-3.7; p=0.035) on multivariable analysis after adjusting for age at symptom onset, sex, time from onset to diagnosis, and site of onset. Multivariable analysis of patients with ALS and behavioural variant FTD showed that the presence of the repeat expansion was associated with a hazard ratio of 3.7 (95% CI 1.1-12.3; p=0.034).

3 T magnetic resonance grey-matter voxel-based morphometry was assessed on a cohort of ten patients with ALS and the repeat expansion and 30 age-matched and disease-duration-matched patients with ALS without the repeat expansion (webappendix p 1). Significant differences in grey-matter atrophy were identified in the cohort with the repeat expansion in the following brain regions: right inferior frontal gyrus, right superior frontal gyrus, left anterior cingulated gyrus, and the right precentral gyrus (figure 2).

Figure 3: Kaplan-Meier survival probabilities for patients with amyotrophic lateral sclerosis stratified for the presence of the C9orf72 hexanucleotide repeat expansion

Kaplan-Meier survival probabilities for all patients with amyotrophic lateral sclerosis (ALS) in the population-based cohort (201 patients, A) and for the subgroup of patients with behavioural variant frontotemporal dementia (25 patients, B).

Predictive modelling determined which factors were associated with presence of a repeat expansion. Of the
Clustering of significant grey matter atrophy in a cohort of ten patients with amyotrophic lateral sclerosis (ALS) and the pathological expanded C9orf72 hexanucleotide repeat compared with 30 matched patients with ALS without the repeat expansion. The four columns show the four clusters (from left to right): right inferior frontal gyrus, right superior frontal gyrus, left anterior cingulate gyrus, and the right precentral gyrus in the three main radiological planes in each row (top to bottom: axial, coronal, sagittal). R-right hemisphere. L-left hemisphere.

191 patients for whom phenotypic data were available, 79 did not have complete behavioural data available from the first assessment and so 112 were included in the modelling analysis. The final model contained the following predictors associated with the presence of the repeat expansion in patients with ALS: a family history of ALS or FTD yielding an odds ratio of 102.2 (95% CI: 3.845-3.845; p = 0.0001); a family history of ALS (defined as at least one first-degree or second-degree relative with ALS) with an odds ratio of 30.8 (95% CI: 6.8-138.5; p < 0.0001); and abnormal behaviour (behavioural impairment was defined as a t-score of 65 or more in at least two subscales of the Frontal System Behavioural Scale) at first assessment, which generated an odds ratio of 4.9 (95% CI: 1.2-19.9; p = 0.027).

A clinical screening algorithm (figure 3) was developed using the predictive factors that had been generated. The algorithm was then applied to the clinical cohort of patients, for whom data was available, to calculate the sensitivity and specificity of the screening method. If genetic testing was offered to all patients with a family history of ALS or FTD, and also to those patients without a family history but with behavioural impairment, and not to those with no family history of ALS or FTD and no behavioural impairment, the sensitivity of this screening algorithm was 100% (95% CI: 0.85-1.00) and the specificity was 67% (95% CI: 0.57-0.76). The high sensitivity of the screening algorithm is due to the fact that no patients...
with normal cognition and behaviour, who had a negative family history, had the repeat expansion (63 patients).

**Discussion**

We have shown that patients with ALS and the C9orf72 hexanucleotide repeat expansion represent a recognisable subphenotype characterised by a lower age of onset, presence of cognitive and behavioural impairment, specific neuroimaging changes, a strong family history of neurodegeneration, and reduced survival. In our cohort, the repeat expansion was not present in patients who had sporadic ALS and no behavioural abnormalities. Our findings show that detailed phenotyping and careful characterisation within a population-based cohort can rapidly generate an algorithm that could potentially be clinically useful in the context of new genetic discoveries (panel).

All patients with the repeat expansion who had been genotyped carried a portion of the 20-SNP consensus haplotype reported by Mok and colleagues, confirming that the C9orf72 hexanucleotide expansion in the Irish population is probably derived from the same founder as other European populations. Many recent discussions of ALS and FTD have suggested that these disorders form two ends of a disease spectrum. However, detailed cognitive assessment of our 191 population-based incident cases suggests that patients with ALS are clinically heterogeneous, and can be divided into at least two broad categories: patients with pure sporadic ALS (no cognitive or behavioural impairment and no reported family history of ALS or FTD), and patients with a predominance of executive cognitive impairment and behavioural change.

We have shown that a high proportion of the latter group carry the repeat expansion. Carriers had a younger age at disease onset, more rapid disease progression, and shorter survival than did non-carriers. This finding has important implications for stratification of patients in clinical trials; it has potential implications for drug efficacy, although our preliminary work has shown no difference in the survival effects of riluzole in patients with the expansion compared with those without (data not shown).

Patients with the repeat expansion also had substantial non-motor cortex changes on high-resolution 3 T structural MRI, and reduced grey-matter volume. These changes correlated with the extensive neuropsychological findings in patients with the repeat expansion, which included increased apathy, increased dysexecutive behaviour, and some evidence of worsened executive function, especially on the Stroop interference task, phonemic verbal fluency, and Brixton spatial anticipation task. Apathy has been consistently linked with the anterior orbitofrontal cortex and the anterior cingulate gyrus, as have the Stroop interference task and the verbal fluency task. Behavioural change was also a prominent feature in patients with the repeat expansion. More extensive imaging and autopsy studies will be needed to characterise in detail the structural and neuropathological differences between patients with and without the repeat expansion. Nevertheless, the prominence of cognitive and behavioural impairment in patients with the C9orf72 repeat expansion has implications in the development of clinical-care pathways and also for education and support of carers, because cognitive and behavioural changes affect patients' ability to adhere to symptomatic treatments, including non-invasive ventilation, and can increase carer burden.

We have shown that a positive family history of ALS or FTD had the highest predictive values for the presence of the repeat expansion. This finding highlights the importance of an accurate and detailed family history. In our cohort, a small proportion of patients originally categorised as sporadic were subsequently shown to have familial ALS on the basis of extensive pedigree interrogation. Identification of FTD in preceding generations is challenging, as clinical recognition of the disorder is relatively recent. We sought to resolve this problem by detailed face-to-face semi-structured interviews with at least two family members, coupled with chart review and death certification, and applied stringent criteria for posthumous diagnosis of FTD. These findings have implications for the current operational definition of familial ALS. Although no consensus exists, most investigators judge familial ALS to include at least one first-degree or second-degree relative with ALS. Our data suggest that a family history of FTD should also be included in the revised definition of familial ALS.

Our detailed family studies suggest that ALS associated with the C9orf72 repeat expansion is probably an autosomal dominant disorder with an expanded phenotype of neurodegeneration and variable penetrance. Patients with a negative family history of neurodegeneration, who have normal cognition and behaviour,
are extremely unlikely to harbour the repeat expansion. Validation of the clinical screening algorithm will be necessary in a larger cohort.

Testing for the presence of the repeat expansion in the at-risk group (ie, patients with evidence of cognitive and behavioural impairment or a family history of neurodegenerative disease) outside of a research setting should be undertaken with caution. Diagnostic testing demands a high degree of certainty, and because of the extensive implications for both patients and their family members, stringent quality control is needed. At present, the precise cutoff for a pathogenic expanded number of repeats remains unclear, and variability exists in the maximum repeat number identified in control populations. Moreover, because the maximum size of the pathological expansions cannot be determined using repeat-primed PCR, formal diagnostics will require definitive validation using Southern blotting.

Whether presymptomatic family members should be offered testing is also unclear. Insufficient data are available to predict the probability that an asymptomatic person with the expansion will develop disease, nor can the likely phenotype be predicted. While presymptomatic testing would be valuable in a research setting, underpinned by strict ethical guidelines, a larger body of research will be required to sufficiently address important issues such as the degree of penetrance, the probability of an expansion from one generation to the next, and patterns of inheritance.

Our study is limited by the size of the cohort with the repeat expansion, although the larger size of the unaffected cohort provides statistical power. Detailed studies of cognitive and family history are time consuming for both the investigator and the patient, and attrition rates are high in longitudinal studies. Nevertheless, our findings provide strong evidence that the C9orf72 repeat expansion is almost exclusively autosomal dominant with variable penetrance, and is associated with a characteristic cognitive and behavioral phenotype and shorter survival. These findings will require verification both in larger cohorts and by detailed neuropsychological, imaging, and autopsy studies. Our study is also limited by the use of repeat-primed PCR rather than Southern blotting. We have not presented any data relating to maximum size of the pathological expansion because of the limited ability of reverse prime-PCR to accurately assess size beyond 60 repeats. Further studies correlating the size of the expansion with clinical phenotype are needed. Also, although we have shown evidence of incomplete disease penetrance, generation of an accurate estimate of the penetrance of the pathological variant has not been possible, because we do not have data relating to the C9orf72 status of unaffected family members. Further studies of larger kindreds, supported by genotyping, will be required to calculate the true penetrance of this variant.

Contributions
SB and AS undertook the genetic analysis. SB gathered family data, and did the statistical analysis. SB and OH wrote the report. ME undertook the neuropsychiatric incident study and analysed cognitive data. MJ, PB, and AAC contributed to the writing of the report. PB undertook the MRI and MRT analysis. AS advised on methods for assessment of genetics. CW provided statistical input to the project. BC supported the neuropsychiatric incident study. MJ gathered data on family history. NJ, CO'R, and BK gathered neuropathological data. KK and RLM run the amyotrophic lateral sclerosis (ALS) bank. KK, RLM, and SB advised on aspects of genetics. CL maintains the ALS register. PM contributed to data collection. JP contributed to a portion of the neuropsychiatric incident study and gathered some of the neuropathological data. ALB advised on aspects of MRI. NP advised on neuropathological aspects. AAC provided laboratory resources and expertise in genetic testing for expansions in C9orf72 and contributed to writing of the manuscript and to the interpretation and analysis of the data. OH is director of the Irish ALS register, designed all aspects of the study, and wrote and edited the manuscript. All authors reviewed the report before submission.

Acknowledgments
We received funding from the Health Seventh Framework Programme (FP7/2007-2013) under grant agreement number 249867 (EUGENOMOT), the Health Research Board, Research Motor Neuron, and the Irish Motor Neuron Disease Association. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement number 259867. We thank the NIH specialised Biomedical Research Centre for Mental Health, South London and Maudsley NHS Foundation Trust (SLeAM), London, UK, and the Institute of Psychiatry, King's College, London, UK.

References
2. Morita M, Al-Chalabi A, Andersen PM, et al. A focus on clonal expansion: from repeat primed PCR to accurately assess size beyond 60 repeats. Further studies correlating the size of the expansion with clinical phenotype are needed. Also, although we have shown evidence of incomplete disease penetrance, generation of an accurate estimate of the penetrance of the pathological variant has not been possible, because we do not have data relating to the C9orf72 status of unaffected family members. Further studies of larger kindreds, supported by genotyping, will be required to calculate the true penetrance of this variant.

www.thelancet.com/neurology Published online February 3, 2012 DOI:10.1016/S1474-4427(12)70014-5