Designing and implementing an international online case-control study of risk factors for amyotrophic lateral sclerosis (ALS)

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Faculty of Medicine and Health

Sydney Medical School

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STATEMENT OF AUTHENTICATION

This thesis is submitted to the University of Sydney in fulfilment of the requirement for the Degree of Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Signature: Date: 18 February 2019

ABSTRACT

Little is known about the contribution of environmental factors to the etiology of ALS. I have developed an online case-control epidemiological questionnaire that contains 165 questions about risk factors for ALS, called “ALS Quest”. ALS Quest is anonymous and web-based, allowing for rapid collection and analysis of data, and is available in 28 languages. ALS patients and controls are recruited primarily via ALS Associations and social media. 1,177 respondents have to date completed the questionnaire.

The methods used to design ALS Quest have been published, and, using data from the questionnaire, I have investigated the following four topics (with one publication for each topic).

1. Changes in the ratio of index:ring finger lengths, related to raised prenatal testosterone levels, had been suggested to occur in ALS. The large ALS Quest finger length dataset showed that the index:ring ratio was the same in ALS and control respondents, indicating that this widely-reported hypothesis is unlikely to be correct.
2. Personality type could underlie the selection of lifestyle factors that could put people at risk for ALS. Indeed, ALS respondents were found to be more agreeable, less neurotic, more conscientious and more extraverted than controls, which could relate, for example, to a greater tendency to smoke (a postulated risk factor for ALS). These findings can also explain the frequent observation that people with ALS are particularly “nice”. Personality could also potentially be linked to ALS via one or more genetic variants.
3. Many clinicians and people with ALS consider that stress could be a trigger for the disease. On the contrary, our ALS respondents did not have more stress-inducing life event or occupational stressors, and were more resilient, than controls. Resilience is largely genetically determined, so this opens a new avenue for ALS research.
4. Mercury has long been suspected to be a neurotoxin that could contribute to ALS. However, common sources of mercury exposure (consumption of fish and number of mercury-containing dental fillings) were similar in ALS and control groups. One item of interest is that ALS respondents consumed more shellfish, which are high in BMAA, a postulated risk factor for ALS.

In summary, ALS Quest has proved to be a valuable resource to study risk factors in ALS and will continue to recruit respondents for international comparisons of risk factors.

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To paraphrase, no person doing their PhD is an island, and there are many people who helped me in innumerable ways along this journey. First and foremost, I would like to acknowledge and thank my supervisor, Roger Pamphlett, who was the inspiration behind this project, and gave me guidance and support throughout. He is the very definition of a scholar and a gentleman, and essentially always knows exactly the right thing to say or do. I have very much enjoyed being his student. My associate supervisor, Susan Hayes, was an invaluable resource on this project, and provided many constructive comments in the development of the research analyses. I also thank Min-Xia Wang for her gracious assistance with ALS Quest, in particular for providing translation assistance for our Chinese version of the questionnaire. Thank you to my laboratory compatriot Stephen Kum Jew for being one of the cheeriest people I know – I will miss our chats over a nice cuppa on the 7th floor. I would also like to thank the entire Neuropathology Department at the Brain and Mind Centre, and in particular, Michael Buckland, for making me feel right at home in their group and for being a lovely group of colleagues. I very much appreciated having a desk of my own in the department, and having others around made this process less isolating.

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around the world, and a large number of the responses received from ALS patients are due to your specific outreach efforts. Thank you for being a part of this.

I also thank the many Rotary groups that gave me the opportunity to come talk with them about ALS Quest and to measure their fingers. I had a wonderful time exploring the Sydney suburbs and meeting with you all.

Thank you to Qualtrics for creating a quality survey software that has allowed us the platform to develop ALS Quest, and for providing some of the best technical support in the business.

Thanks to my wonderful family – my mom, my dad, Jens, Ellie, and Henry – for supporting me through this journey and giving me the space and time needed to do this work.

Last but not least, a shout out to Karilyn Heisen, who provided the spark that led to this whole endeavor.

To the many ALS patients I had the fortune to meet along this journey – please keep hoping and remembering. We are all in this fight with you, and I am honored to be a part of it.

LIST OF PUBLICATIONS

1. Chapter 2 of this thesis is published as: **JA Parkin Kullmann**, S Hayes, MX Wang, R Pamphlett. Designing an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study. *JMIR Res Protoc* 2015; 4, e96.

I co-designed the study with the co-authors and wrote the first draft of the manuscript.

2. Chapter 3 of this thesis is published as: **JA Parkin Kullmann**, R Pamphlett. Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case–control study. *BMJ Open* 2017; 7, e0169.

I co-designed the study with the co-author, analysed the data and wrote the first draft of the manuscript.

3. Chapter 4 of this thesis is published as: **JA Parkin Kullmann**, S Hayes, R Pamphlett. Are people with ALS really nicer? An online international study of the big five personality traits. *Brain Behav* 2018; 8, e01119.

I co-designed the study with the co-authors, analysed the data and wrote the first draft of the manuscript.

4. Chapter 5 of this thesis is published as: **JA Parkin Kullmann**, S Hayes, R Pamphlett. Is psychological stress a predisposing factor for amyotrophic lateral sclerosis (ALS)? An online international case-control study of premorbid life events, occupational stress, resilience and anxiety. *PLoS One* 2018; 13: e0204424.

I co-designed the study with the co-authors, analysed the data and wrote the first draft of the manuscript.

5. Chapter 6 of this thesis is published as: **JA Parkin Kullmann**, R Pamphlett. A Comparison of Mercury Exposure from Seafood Consumption and Dental Amalgam Fillings in People with and without Amyotrophic Lateral Sclerosis (ALS): An International Online Case-Control Study. *Int J Environ Res Public Health* 2018; 15, 2874.

I co-designed the study with the co-author, analysed the data and wrote the first draft of the manuscript.

INTERNATIONAL AND NATIONAL CONFERENCE PRESENTATIONS

JA Parkin Kullmann. "ALS Quest: Initial Results from an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Motor Neurone Disease," poster presentation at MND Australia Research Meeting in Sydney, Australia, 22-23 November 2015.

JA Parkin Kullmann. "Designing and Implementing an International Web-Based Questionnaire to Look at Risk Factors for ALS/MND," platform presentation at the 25th Annual Meeting of the International Alliance of ALS/MND Associations in Boston, Massachusetts, 5 December 2017.

JA Parkin Kullmann and R Pamphlett. "Designing and implementing an international web-based questionnaire to look for risk factors for ALS/motor neuron disease," poster presentation at 28th International Symposium on ALS/MND in Boston, Massachusetts, 8 December 2017.

JA Parkin Kullmann and R Pamphlett. "Do people with ALS have lower index-to-ring finger length ratios (2D:4D)?" poster presentation at 28th International Symposium on ALS/MND in Boston, Massachusetts, 8 December 2017.

JA Parkin Kullmann, R Pamphlett, and S Hayes. "Are people with ALS really nicer? An online international study of the big five personality traits," poster presentation at 28th International Symposium on ALS/MND in Boston, Massachusetts, 8 December 2017.

JA Parkin Kullmann and R Pamphlett. "Is psychological stress a predisposing factor for ALS? An online international case-control study of premorbid stressful life events, resilience and anxiety," platform presentation at 29th International Symposium on ALS/MND in Glasgow, Scotland, 8 December 2018.

JA Parkin Kullmann and R Pamphlett. "Is exposure to mercury a risk factor for ALS? Analysis of results from an international online case-control study," poster presentation at 29th International Symposium on ALS/MND in Glasgow, Scotland, 8 December 2018.

PERMISSION FROM CO-AUTHORS

In addition to the statements in the following three pages, in cases where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author.

Jane A Parkin Kullmann Date 18 February 2019

As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements in the following three pages are correct.

Roger Pamphlett Date 18 February 2019

The University of Sydney
Sydney Medical School
Sydney, Australia, 2006

To Whom It May Concern,

We the undersigned are writing this letter to document the role of Jane A. Parkin Kullmann in the preparation and submission of the following manuscript.

Parkin Kullmann JA, Hayes S, Wang MX, Pamphlett R. Designing an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study. JMIR Res Protoc 2015;4:e96
https://www.researchprotocols.org/2015/3/e96/

Jane A. Parkin Kullmann, during her PhD candidature, performed the following tasks for this paper: developing the questionnaire, performing background research, writing the draft manuscript, and responding to reviewer's comments.

The individual roles of co-authors are listed below:

Task	Co-author Role
Questionnaire development	JPK, RP, SH, MW
Background research	JPK, RP, SH
First draft of manuscript	JPK
Editing and submission of manuscript	RP, JPK, SH

Sincerely,

Roger Pamphlett

Susan Hayes

Min-Xia Wang

The University of Sydney
Sydney Medical School
Sydney, Australia, 2006

To Whom It May Concern,

We the undersigned are writing this letter to document the role of Jane A. Parkin Kullmann in the preparation and submission of the following manuscript.

Parkin Kullmann JA, Hayes S, Pamphlett R. Are people with amyotrophic lateral sclerosis (ALS) particularly nice? An international online case–control study of the Big Five personality factors. Brain Behav 2018;e01119 https://doi.org/10.1002/brb3.1119

Jane A. Parkin Kullmann, during her PhD candidature, was involved in the study design, managed the questionnaire data, conducted statistical analyses of the data, wrote the draft manuscript, and responded to reviewer's comments on the manuscript.

The individual roles of co-authors are listed below:

Task	Co-author Role
Study design and conceptualisation	JPK, RP, SH
Data management and statistical analysis	JPK, RP
First draft of manuscript	JPK
Preparation and editing of manuscript	RP, JPK, SH

Sincerely,

Roger Pamphlett

Susan Hayes

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Sydney, Australia, 2006

To Whom It May Concern,

We the undersigned are writing this letter to document the role of Jane A. Parkin Kullmann in the preparation and submission of the following manuscript.

Parkin Kullmann JA, Hayes S, Pamphlett R (2018) Is psychological stress a predisposing factor for amyotrophic lateral sclerosis (ALS)? An online international case-control study of premorbid life events, occupational stress, resilience and anxiety. PLoS ONE 13: e0204424 https://doi.org/10.1371/journal.pone.0204424

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Data management and statistical analysis	JPK, RP, SH
First draft of manuscript	JPK
Preparation and editing of manuscript	RP, JPK, SH

Sincerely,

Roger Pamphlett

Susan Hayes

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Chapter 1: General Introduction

1.1 Context

Amyotrophic lateral sclerosis (ALS) (also called motor neuron disease or MND) is a neurodegenerative disease with a largely unknown pathogenesis. It is generally accepted that in approximately 10 percent of ALS patients the disease has a primarily genetic basis (familial ALS), and for the remainder it is considered sporadic (or isolated) ALS, typically occurring in only one member of a family and without a known cause [1]. This lack of information regarding how and why ALS arises makes it an eminently suitable subject for toxicological and epidemiological research. This thesis partially addresses this knowledge gap by investigating novel environmental and lifestyle risk factors for ALS, using data collected from an international online epidemiological questionnaire that compares the exposures of cases (ALS respondents) and non-ALS controls.

1.2 Orientation to Thesis

This introductory chapter describes the research problems investigated and summarises the objectives and aims of this project. Chapters 2 through 6 are published papers that are presented as components of the thesis, with each chapter being preceded by a brief introduction. The final chapter (Chapter 7) is a discussion of the implications of this research and ideas for future exploration. The Appendices to the thesis contain i) a copy of ALS Quest, the epidemiological case-control questionnaire developed as a part of this thesis; ii) supplementary research findings; and iii) copies of poster and oral presentations given at conferences.

1.3 Challenges in ALS Research

The issue of external causal factors for ALS has been explored in depth by the medical and epidemiological community primarily beginning in the 1970s [2,3]. The factors most commonly implicated by these studies as contributors to ALS are (in no particular order) exposures to toxicants such as cyanobacteria, mercury, lead, pesticides, and air pollution; smoking; histories of athletic activity or exercising; and occupational exposures [4]. The most voluminous and perhaps persuasive research to date has focused on pesticides, athletic

participation or physical activity, heavy metals, and smoking. However, little consensus exists regarding which of the many possible environmental exposures and lifestyle choices are in fact risk factors for the disease. As a result, clinicians are unable to either offer advice about how people might either prevent ALS, or why people who are diagnosed with ALS might have developed the disease.

This lack of validated risk factors is most likely a reflection of the complexity of the process by which ALS arises. In the model of ALS pathogenesis proposed by Al-Chalabi and Hardiman [5], it was suggested that a genetic component exists before birth, followed by a combination of cell damage and environmental exposures over time, that, once it reaches a threshold, leads to the onset of the disease. Therefore, rather than any one environmental or lifestyle factor serving as the ALS progenitor, a number of them in combination are likely to precipitate the disease. In a subsequent analysis, Al-Chalabi and others estimated that the development of ALS is a six-step process [6], implying that six distinct environmental exposures or lifestyle factors are needed to cause the disease. For those with a genetic predisposition to ALS, between two and four steps may be needed to initiate ALS [7].

Given this number of necessary risk factors, and the fact that these factors may vary from individual to individual, large and diverse epidemiological case-control studies are needed to provide adequate power to identify risk factors. Thus far, epidemiological investigations of ALS have primarily been conducted using paper-based questionnaires, which are typically time- and resource-intensive. In addition, previous case-control questionnaire-based ALS epidemiological projects have had other limitations which make them incomplete. These include small sample size with limited numbers of questions, time pressure on respondents, the effort needed for data transcription and data entry from paper-based-questionnaires, and being based in a single country. Results from only one other known online ALS questionnaire (regarding muscle cramps in ALS) have been published [8], despite the wide acceptance of web-based questionnaires in the epidemiological community [9]. Another challenge in conducting epidemiological studies is that ALS has a short course, with a median survival time of three years [1], so prompt and comprehensive ascertainment of ALS patients is critical for data collection.

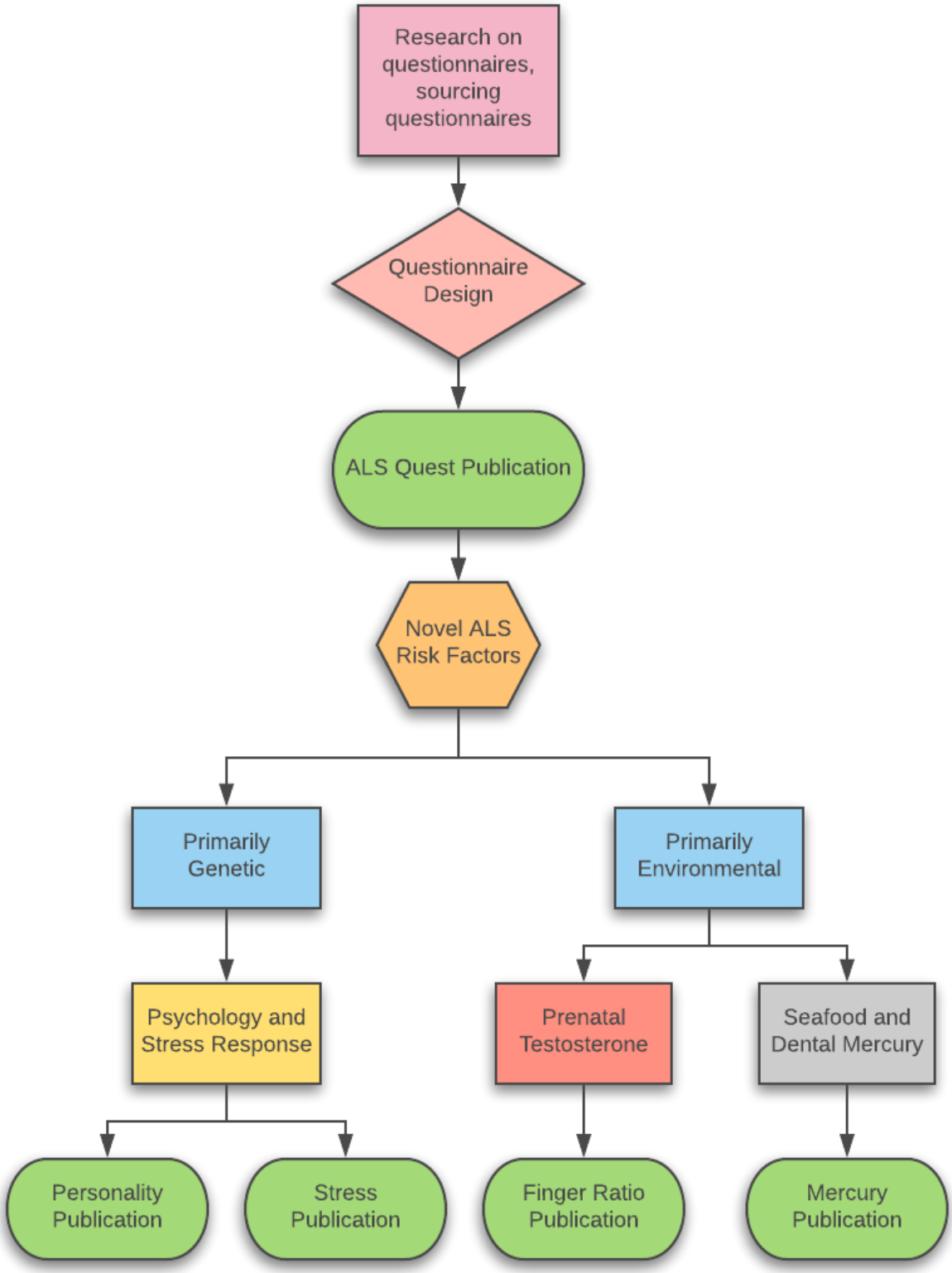
1.4 Objectives of the Project

Given the challenges outlined above, the objectives and aims of this project were threefold:

1. To create a comprehensive ALS epidemiological case-control questionnaire encompassing both previously-studied ALS risk factors, as well as novel risk factors.
2. To base the questionnaire in an online platform, so that it was immediately accessible to a large number of people. This reduced the level of effort for data entry and analysis and allowed for the questionnaire to be available in many languages.
3. To use the data collected from this questionnaire to compare various environmental exposures and lifestyle aspects between ALS respondents and controls.

The development of this thesis is summarised in the below Figure 1-1.

Figure 1-1. Thesis Flowchart



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Chapter 2: Designing an internationally accessible web-based questionnaire to discover risk factors for amyotrophic lateral sclerosis

Epidemiological studies of ALS have most often been conducted using traditional tools, such as paper-based questionnaires. However, these methods have their limitations – primarily, that data collection can be slow and data transcription can be laborious. This study overcame these limitations by using an online case-control epidemiological questionnaire, the chief benefit being that responses can be collected much more rapidly, which is critical in a disease such as ALS where the course is typically short.

The preparation of the questionnaire was undertaken over a year because of its critical importance as the platform upon which the remainder of the analyses rested - creating an appropriate questionnaire was key to the success of the project. In addition, as the first major online ALS epidemiological questionnaire, it was important to create a robust and valid instrument to show that online collection of epidemiological data was suitable for a disease like ALS. Another goal was to produce a truly international questionnaire, so the questionnaire was translated by volunteers into 27 languages other than English.

A significant part of the development of ALS Quest involved the revision and expansion of a prior paper-based ALS questionnaire [1-7] used by the Australian MND DNA Bank for use in the new online format. The updated questionnaire also included several topics sourced from previously published ALS questionnaires, with further questions added to address heretofore unexplored ALS risk factors.

The following article included in Chapter 2 provides an overview of the methods used in this research project, including considerations made when designing the questionnaire, the resources used in compiling the questions, information about how the questionnaire was implemented and distributed, how participants were recruited, and how data from the questionnaire were managed. The article closes by providing a brief overview of the questionnaire results at that time, as well as a summary of the limitations of a web-based questionnaire approach.

A copy of the complete ALS Quest research questionnaire is included as Appendix A.

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Original Paper

Designing an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with a typical survival of three to five years. Epidemiological studies using paper-based questionnaires in individual countries or continents have failed to find widely accepted risk factors for the disease. The advantages of online versus paper-based questionnaires have been extensively reviewed, but few online epidemiological studies into human neurodegenerative diseases have so far been undertaken.

Objective: To design a Web-based questionnaire to identify environmental risk factors for ALS and enable international comparisons of these risk factors.

Methods: A Web-based epidemiological questionnaire for ALS has been developed based on experience gained from administering a previous continent-wide paper-based questionnaire for this disease. New and modified questions have been added from our previous paper-based questionnaire, from literature searches, and from validated ALS questionnaires supplied by other investigators. New criteria to allow the separation of familial and sporadic ALS cases have been included. The questionnaire addresses many risk factors that have already been proposed for ALS, as well as a number that have not yet been rigorously examined. To encourage participation, responses are collected anonymously and no personally identifiable information is requested. The survey is being translated into a number of languages which will allow many people around the world to read and answer it in their own language.

Results: After the questionnaire had been online for 4 months, it had 379 respondents compared to only 46 respondents for the same initial period using a paper-based questionnaire. The average age of the first 379 web questionnaire respondents was 54 years compared to the average age of 60 years for the first 379 paper questionnaire respondents. The questionnaire is soon to be promoted in a number of countries through ALS associations and disease registries.

Conclusions: Web-based questionnaires are a time- and resource-efficient method for performing large epidemiological studies of neurodegenerative diseases such as ALS. The ability to compare risk factors between different countries using the same analysis tool will be of particular value for finding robust risk factors that underlie ALS.

(JMIR Res Protoc 2015;4(3):e96) doi:10.2196/resprot.4840

KEYWORDS

amyotrophic lateral sclerosis (ALS); motor neuron disease (MND); web-based; online; questionnaire; epidemiology; risk factor; case-control study; international; language translation

Introduction

Amyotrophic lateral sclerosis (ALS, also known as motor neuron disease or MND) is a progressive neurodegenerative disease of adults with a usual survival of three to five years after diagnosis [1]. Epidemiological studies using traditional methods of collecting data via mailed paper questionnaires or via telephonic or in-person interviews have so far not revealed any widely accepted environmental or lifestyle risk factors for ALS.

Previous epidemiological studies of ALS have had a number of limitations. ALS has an incidence of about 2-3 per 100,000 in most populations, so it is not a common disorder and obtaining large numbers of respondents has been difficult [2]. No intercountry comparisons of risk factors for ALS using the same survey tool have been undertaken. Restricting the geographical region of recruitment to one country or continent prevents identification of risk factors that vary across countries [3] or ethnic groups. As new criteria to classify ALS into its sporadic and familial forms are proposed, changing diagnostic criteria will make characterisation of cases in previous studies difficult [4]. In addition, new potential environmental risk factors for ALS are continually being proposed, but it is inconvenient to add questions to non-Web surveys.

We became aware of these and other limitations of paper-based questionnaires during the course of an Australian study looking for risk factors for sporadic ALS. Despite this being a continent-wide survey undertaken over 11 years (2000-2011) with active recruitment of participants by state-based ALS associations, responses were obtained from only 812 ALS patients and 793 nonrelated controls in a population of 23 million people. Although this remains one of the largest epidemiological case-control databases in ALS with several publications arising from the study [2,3,5-10], numbers were too small to analyse subgroups in many categories, such as those for less common occupations. The majority of respondents were English-speaking and of western European descent although people from many language groups live in Australia (some of this bias can be explained by the questionnaire being available only in English). The criteria we used for separating familial and sporadic ALS are under revision, and many of the patients we classified as having familial ALS would now be considered to be in the sporadic group [4]. The financial cost to obtain and process information was high and when funding for staff and consumables came to an end, the survey had to close. We did not ask questions about topics such as psychiatric conditions since the respondents had to identify themselves, and this understandably would have made many reluctant to give out such information.

Many of the limitations experienced during our paper-based study have been overcome by migrating to an online questionnaire, where respondents are not asked for personally identifiable information. Our Web-based questionnaire can be easily translated into other languages for both reading and answering questions, which will aid recruitment and allow for international comparisons of risk factors. We describe our approach to designing this online questionnaire to look for risk factors in ALS and present the initial responses to this survey. We also summarise the advantages and disadvantages of Web-based versus paper-based questionnaires as they pertain to looking for risk factors for neurodegenerative diseases.

Methods

Questionnaire Software

Questionnaire platforms from a number of providers were evaluated as potential sources of Web-based survey software. Most offered user-friendly survey design, secure storage of respondent data, an online log-in portal that allows users to access the survey from any Internet browser, and the ability to download survey data in several formats. Qualtrics [11] was identified as a good platform for our project because it is flexible and provides a large diversity of question types.

Questionnaire Design

Overview

Relevant questions from our original paper-based ALS questionnaire were entered into the Qualtrics platform using the appropriate question formats (see Multimedia Appendix 1 to view the original paper-based questionnaire). The design of our online questionnaire was based on recent recommendations of best practice in this field [12-14].

Pay Careful Attention to the Wording of Questions to Ensure Clarity

Our experience with our previous paper-based questionnaire was helpful in identifying types of questions that tended to result in ambiguous answers.

Use Predetermined Choices to Ensure Standard Answers

For example, questions requiring a written answer in a paper-based questionnaire (eg, “In which country are you currently living?”) can be formatted as a single-choice drop-down menu in a Web-based format. The number of answers requiring text entry, which can cause transcription difficulties and delay access to the data, was reduced to a minimum in the online questionnaire.

Place Questions Into Topic Groups

The online questionnaire is organised according to topics of interest (eg, occupation, exercise). This improves coherence of the questionnaire, and it also allows easier topics to be placed towards the beginning of the survey to increase respondents’ confidence about entering data into the questionnaire.

Use Automated Question Logic

Question logic shows or skips certain questions based on previous answers. This relieves respondents of the responsibility of following the logic of a paper-based questionnaire, and ensures they only need view questions that apply to them. Question logic largely eliminates commission errors (ie, answering questions that are not applicable) and omission errors (ie, not answering questions that are applicable) [14]. Question logic applies to about 25% of our online questions.

Avoid Use of a Progress Bar

A progress bar, which shows respondents how far into the survey they are, was not used. First, a progress bar would have been misleading because it does not take into account the show/skip logic within the questionnaire. Second, a progress bar is not recommended on longer surveys because it discourages completion [14].

Access for Patients With Physical Disabilities

Access to the questionnaire was a concern given that respondents with ALS could have limited mobility. We therefore ensured the questionnaire is compatible with speech-to-text programs

and spoken commands. To aid visibility, we set the default font size at 12 point, made the text of all questions in bold font, and implemented a software feature that highlights the question being worked on.

Access in Different Languages

We plan to translate the questionnaire into many languages, including all languages spoken in countries within the International Alliance of ALS/MND Associations. Respondents will select their preferred language from a list of available translations before entering the questionnaire. For text entry, respondents will be able enter answers in their own language. Since only a few questions are answered by entering text, translations to English will not be onerous.

Google Translate is used to perform the first rough translation of non-English languages, but fluent speakers of both English and the language to be translated need to spend many hours amending this to obtain the correct meaning and grammar in the text, based on the English version. For example, in our question about skin color, the word *fair* in most languages is translated as *reasonable* rather than the intended meaning of *light in color*. Qualtrics has a function in which the English and Google-translated non-English version of the questionnaire can be presented side-by-side, so the translator can readily edit the non-English version with reference to the meaning in the English version.

We have chosen first to check and adjust the translation of simplified Chinese, one of the languages where Google Translate appears to give the greatest number of ambiguities. Fluent speakers of other languages are in the process of checking other Google translations. The Google-translated languages that have been checked for accuracy (only simplified Chinese at the time of manuscript submission) will be indicated in the language list as available translations.

New Content in the Web-Based Questionnaire

General

The content of our paper-based questionnaire was compared to the Stanford University ALS Consortium of Epidemiologic Studies (ACES) questionnaire [15], and questions were added or modified on topics such as alcohol and tobacco use, medical history, hobbies and pastimes, and pesticide and chemical exposures. The differences in our paper- and Web-based questions can be viewed by comparing the paper-based questionnaire in Multimedia Appendix 1 and the online questionnaire [16].

Defining Familial Versus Sporadic ALS

Controversy persists as to the definition of familial versus sporadic (or isolated) ALS, with some clinicians classifying a patient as having familial ALS only if close family members also have the disease [4]. Based on studies of the heritability of familial ALS, the questionnaire now asks for the number of first-, second-, and third-degree relatives as well as more distant relatives who have ALS [4,17,18]. It further asks for the total number of first-, second-, and third-degree relatives in the respondent's family overall, since the familial nature of a disease is harder to detect in a small family. Having this detailed family history will allow researchers who have access to our survey data to use their own criteria to define familial and sporadic ALS.

Dementia

Questions are now asked about the number of family members diagnosed with frontotemporal dementia (FTD), a recently recognised component of an ALS/FTD disease continuum [19]. This will allow our study to identify families where one member has ALS while another has FTD.

Genetic Variants

We now ask whether any ALS patient or relative has been identified as having a genetic variant associated with ALS. We do not ask respondents to identify the particular genetic variant since rare variants could constitute personally identifiable information.

ALS Functional Status

People with ALS are asked to complete the ALS Functional Rating Scale (ALS-FRS) [20] to assess their physical state at the time of taking the questionnaire. This will allow an assessment of the rate of progression of the disease, which can be calculated from the time of disease onset. A Web-based format for the ALS-FRS has previously been validated by comparing Web and in-person evaluations [21].

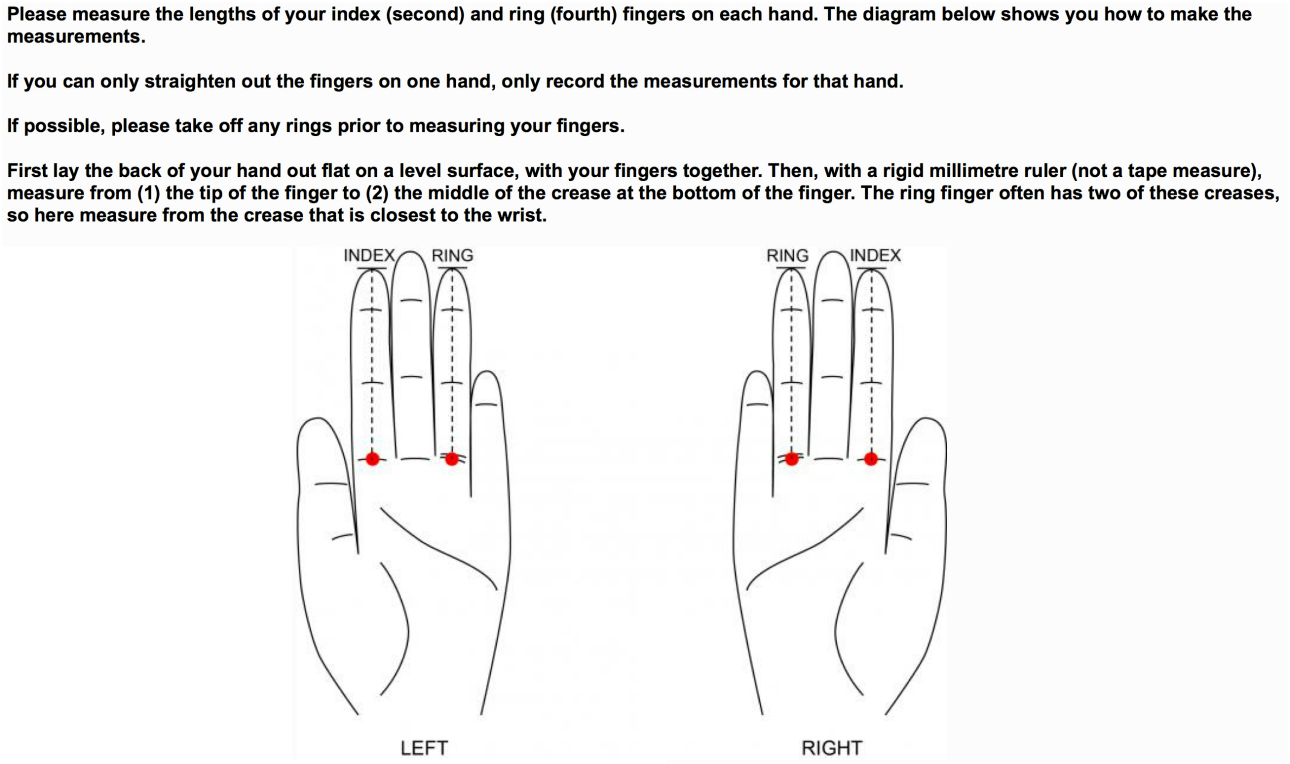
Physical Activity

To evaluate physical activity, which has been suggested to be a risk factor for ALS [22], questions were obtained from surveys used by the European Amyotrophic Lateral Sclerosis Consortium (EURALS) [23] and the European Multidisciplinary ALS Network Identification to Cure Motor Neuron Degeneration (Euro-MOTOR) [22].

Ratio of Finger Lengths

The ratio between the length of the ring finger and index finger, associated with prenatal exposure to testosterone, has been implicated as a risk factor for ALS [24]. A diagram has been included to show respondents how to perform and report these measurements (Figure 1). The reliability of these self-reported finger measurements is currently being investigated by photographing 100 volunteers' hands and comparing their own finger measurements with measurements by researchers using the photographs.

Figure 1. This diagram in the questionnaire shows respondents how to measure the length of their index and ring fingers. Below the question, boxes are provided (not shown) for respondents to select the measurements from a drop-down menu.



Male Pattern Baldness

It has been suggested that men with early-onset alopecia have a higher risk of ALS [25]. We therefore included a question used by the Physicians' Health Study in which men estimate the pattern of any hair loss they may have had when they were 45 years old by selecting one of 5 images ranging from no to marked hair loss [26,27].

Head Trauma

Head trauma has been implicated as a risk factor for ALS [28]. To gauge a history of head trauma, we added questions from the Retrospective Screening of Traumatic Brain Injury (RESTBI) Questionnaire [29].

Sun Exposure

Vitamin D deficiency has been implicated as a factor in ALS [30]. In most countries, sun exposure is the main source of vitamin D [31], but assessing sunlight exposure over long periods of time with a questionnaire is difficult [32]. We therefore asked about two aspects of sun-induced vitamin D generation, skin color and the reaction of the skin to sunlight, as used in the NSW Prostate Cancer Care and Outcomes Study [33].

L-BMAA

Because of the interest in a possible connection between the environmental toxin β-N-methylamino-L-alanine (L-BMAA) and ALS, we included questions related to L-BMAA exposure based on the French BMAALS program questionnaire [34].

Stress

Stress has been suggested as a potential risk factor for ALS [35]. Our questionnaire asks systematic questions about stress as a risk factor for the disease. To assess lifetime stress we used the Social Readjustment Rating Scale which scores the stress associated with a variety of events [36]. To evaluate the likely impact these stressors would have had on respondents, we used the Big Five personality traits assessment [37-39], the Connor-Davidson Resilience Scale [40], and the Geriatric Anxiety Inventory [41,42]. A Web-based administration of a scale similar to the Geriatric Anxiety Inventory has been validated by comparing Web and telephone interview surveys [43].

Diagnosis of ALS

On our previous paper-based questionnaire, we asked neurologists of ALS patients to send us copies of their clinical notes so that the type of ALS the respondent had could be assessed (there are four major types of the disease). This required a consent form specific to Australia, and individual neurologists around the country had to be contacted. No response was received from neurologists for about 15% of respondents, whose questionnaire data could not then be used. Since we designed the current questionnaire to be used for international comparisons of ALS risk factors, a direct approach to neurologists in different countries was not ethically feasible. On the online questionnaire, we therefore ask ALS patients to choose which type of ALS they have been diagnosed with from a predetermined list, and ask them to contact their neurologist or family doctor if they are unsure about the type.

Avoidance of Culturally Specific Questions

All questions were checked for content that could cause misunderstandings in different countries and cultures. We avoided questions that relate specifically to cultural or environmental aspects of any country.

Information for Participants

Text providing information for participants (administrative details about the questionnaire), comprehensive instructions (how to complete the questionnaire), and guidelines (tips for using the questionnaire) appear after respondents access the questionnaire. Respondents then need to answer a few questions before being able to fully access the questionnaire. Respondents are asked to select age, gender, and whether they have ALS. They are asked to describe their connection to ALS if they do not have the disease. If they have a friend or partner with ALS, respondents are asked to list the length of the relationship. Last, respondents are asked how they heard about the questionnaire. After these are answered, an online consent form is displayed; once this is completed, respondents enter the main body of the questionnaire. All other questions are voluntary, but if a question is not able to be answered there is usually an option to explain why (eg, not applicable).

Pairing of Cases and Matched Controls

ALS patients are asked to nominate (if available) a spouse/partner and friends to complete the questionnaire. ALS patients create a unique code and provide it to their spouse/partner and friends. The code is then used to link the ALS patient to these matched controls. This enables paired statistics to be performed on people who are likely to have similar environmental exposures; these statistics will be used for comparisons with nonmatched controls. The code does not allow participants to view other responses.

Questionnaire Distribution

Qualtrics provides two means by which a questionnaire may be distributed: via an anonymous link or via an email invitation with a link specific to each respondent. We chose the anonymous option to maintain participant confidentiality. The questionnaire does not ask for any personally identifiable information such as name, email address, employer name, or exact locations lived. This preserves the anonymity of respondents, which is important considering the sensitivity of some of the data (eg, psychiatric history) being collected. In addition, the anonymous option allows distribution of the questionnaire to a wide international group of potential respondents.

Recruitment of Participants

People both with and without ALS are being sought to complete the questionnaire. The only exclusion criterion is being under the age of 18 years, so there is little possibility for confusion about eligibility criteria. ALS patients in Australia are recruited via newsletters, Facebook pages, and meetings of ALS associations in each state. Nonmatched controls are recruited in particular among community groups such as Rotary International. In the United States, participants are recruited through the government-funded National ALS Registry at the Agency for Toxic Substances and Disease Registry (Centers for Disease Control and Prevention), which has been used by other researchers to recruit participants for ALS online epidemiological surveys [44]. Participants in other countries will be recruited through their respective national ALS associations with the assistance of the International Alliance of ALS/MND Associations.

Data Collection and Storage

Responses to the questionnaire are initially placed on password-protected Qualtrics servers in the countries that host these servers. The Qualtrics servers in the United States are used in countries that do not have their own Qualtrics servers. Completed questionnaire responses are downloaded and transferred from the Qualtrics server into Excel (Microsoft Corporation) and SPSS (IBM Corporation) program files on a regular basis. The original responses are deleted from the Qualtrics servers every six months. Questionnaire responses are kept in a password-protected file on a password-protected computer at the University of Sydney. This computer is connected to Wi-Fi only via password-protected networks.

Results

Cases and Controls

Major groups in the study comprise those who have been diagnosed by a neurologist as having ALS (cases), spouse/partners and friends of people with ALS (matched nonrelated controls), blood relatives of people with ALS who do not have the disease (matched related controls), and persons completing the survey who do not fall into the other categories (nonmatched controls).

The Online Questionnaire

The questionnaire can be viewed online [16]. Examples of multiple choice questions are shown for Single Choice (Figure 2), Select All That Apply (Figure 3), and Drop-Down Menu (Figure 4) questions. An example of a Side-by-Side question is shown in Figure 5.

Figure 2. Example of a single-choice question. Only one choice of place of birth is allowed.

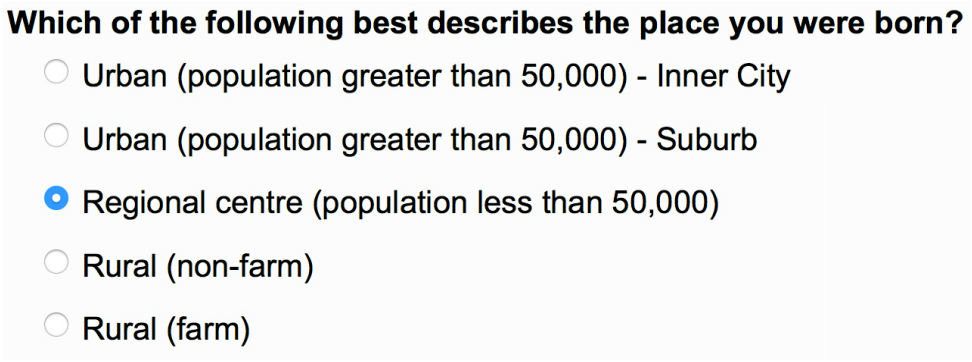


Figure 3. In an all-that-apply question respondents can tick as many answers as they want. In this particular question about occupational exposures there is a possible mix of tick-boxes and script entries.

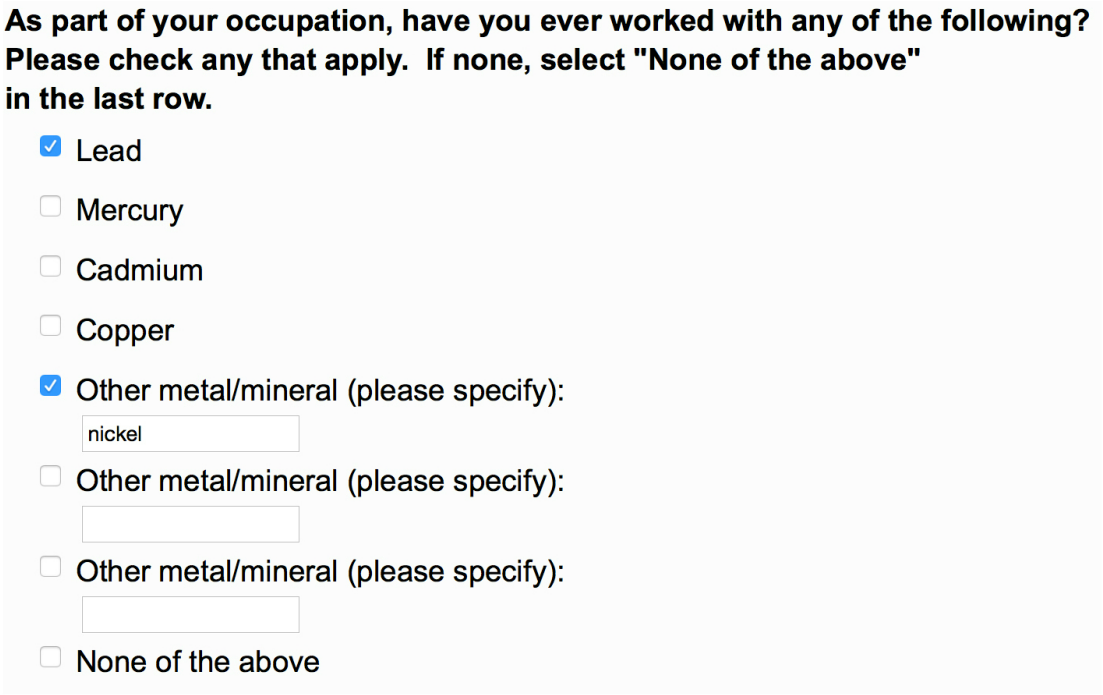


Figure 4. In these three questions about caffeine consumption respondents pick predetermined answers from drop-down lists.

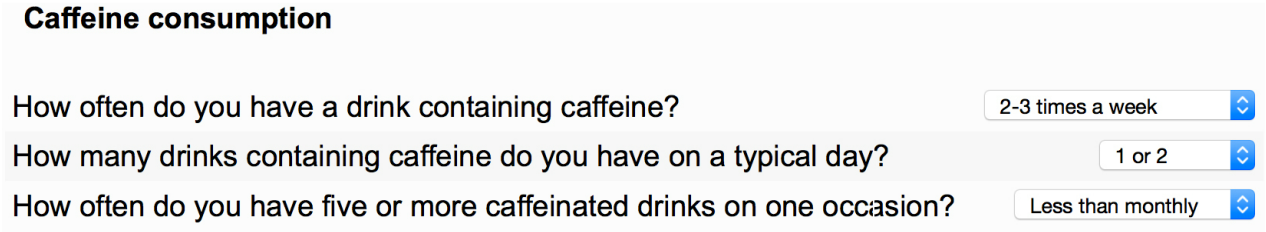
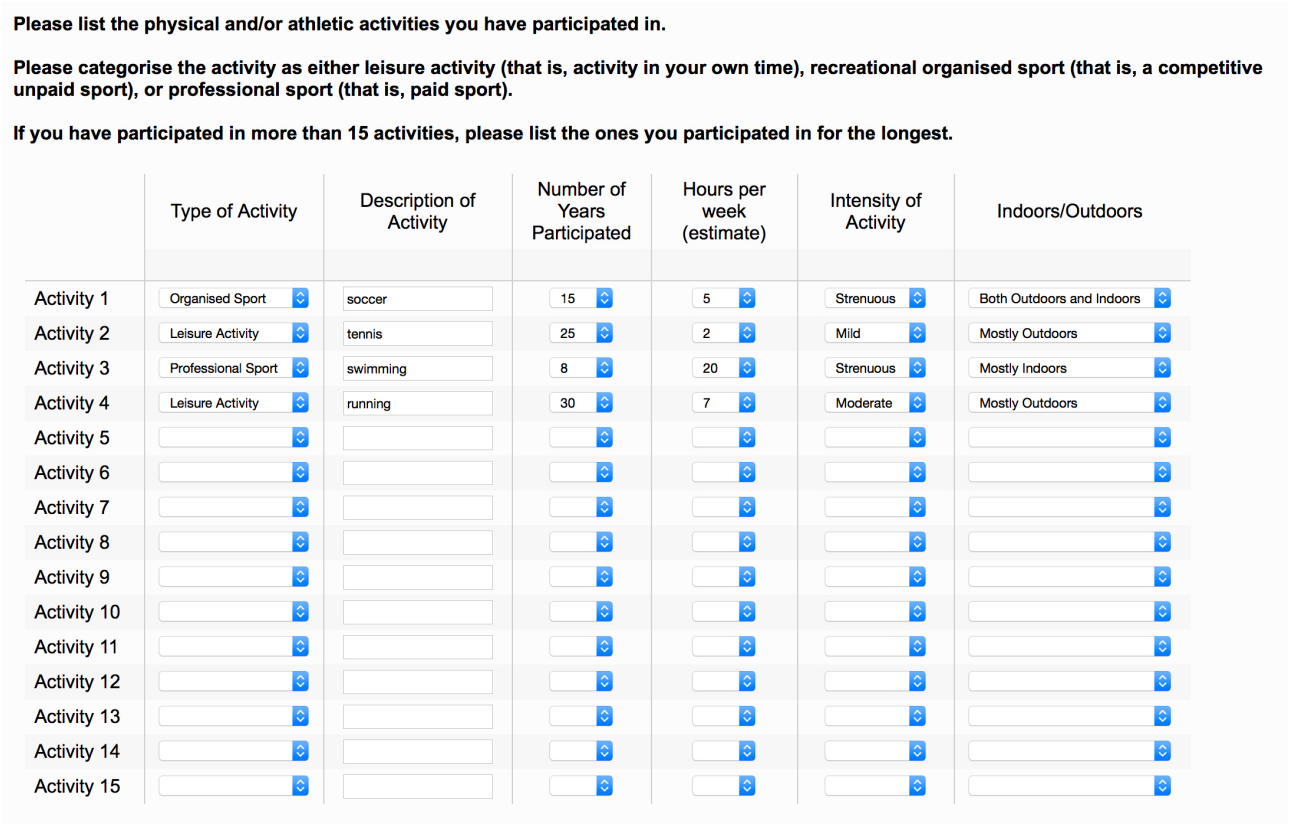


Figure 5. A large amount of information about the type, duration, intensity, and category of an activity can be obtained using side-by-side drop-down menus and script entry.



Pilot Assessment

Ten people were asked to complete the questionnaire and provide feedback to test the clarity of the questions and the functionality of the questionnaire on multiple Internet browsers and devices. Based on this feedback, we adjusted some of the instructions for completing the questionnaire and edited the wording and format of some questions and choices of answers. In addition, after the survey first went online we received email feedback from some of the first 112 respondents. On the basis of this feedback a few minor changes were made and some questions were added. These changes did not affect the validity of the initial 112 responses.

Acceptance and Initial Uptake of the Questionnaire

After approval from an institutional ethics committee, the questionnaire was placed online on 30 January 2015. Four months later, 379 responses (204 from ALS patients and 175 from controls) had been collected. In comparison, after 4 months we had received only 46 respondents from the same population using our paper-based questionnaire.

Spontaneous feedback via email; verbal feedback at meetings of ALS patients (including those with physical disabilities) and their partners; and comments from scientific and medical colleagues concerning the questionnaire format, its content, and ease of use have been positive. However, because we did not formally ask for this information from all respondents this feedback is not quantifiable.

Respondents report taking about two hours to complete the survey, and some appeared to complete it over multiple sessions.

The majority of respondents so far have been from Australia since recruitment from countries outside Australia is in the initial stages. We will be promoting the non-English language versions of the questionnaire as their Google-translated versions are checked.

Ages of Respondents in the Paper- and Web-Based Questionnaires

The average age of the first 379 respondents to the online questionnaire was 54 years (SD 15, range 18-86) compared to an average age of the first 379 respondents to the paper-based questionnaire of 60 years (SD 11, range 28-90).

Discussion

Advantages of Web-Based Questionnaires in Neurodegenerative Diseases

Large numbers of responses can be acquired at low cost with minimal staff requirements and within a short period of time. This is especially relevant to some of the less common neurodegenerative disorders with short survival periods where traditional survey methods have had difficulty recruiting adequate numbers of respondents. Questions can be added easily when newly proposed risk factors are suggested. New risk factors for neurodegenerative diseases are continually being proposed, and with the advent of next generation DNA sequencing, the search for gene-environment interactions underlying these diseases is likely to accelerate. Automatic transfer of response data into database, spreadsheet, and statistics programs virtually eliminates the possibility of transcription errors and speeds up the data analysis. It also reduces the cost

of running these surveys so they can be operated for longer periods, an important consideration when recruiting respondents with rare diseases. Other advantages of Web-based questionnaires have been well documented [45-49].

Studies Comparing Online Versus Other Survey Modes

A review of 29 studies with a combined total of more than 15,000 respondents comparing different survey modes (postal mail, fax, email, and Web-based surveys) reported that Web-based surveys provided a better quality of response, greater level of detail, and greater compliance in answering open-ended questions than mail surveys [50]. The authors calculated similar response rates for the Web-based (52%) and mailed (51%) modes but found that average response times for Web surveys (7 days) were shorter than for mail (17 days). A population survey of 3148 Danish parents concerning their children's health and welfare found similar response rates comparing paper, paper with Web option, Web-only, and Web with incentive formats [51].

The Black Women's Health Study of 59,000 African-American women reported that Web-based surveys were filled out more completely than paper surveys and cost only 25% of paper surveys. Web-based response rates were greatest for younger age groups [52]. In the French NutriNet-Santé study of lifestyle and health, 94% of 147 volunteers stated a preference for the Web-based over the paper version [53]. Furthermore, this study found that the Web-based version prevented the omission of approximately 2% of answers (more than 550 values), which increased the value of each response. It also noted the cost benefits of the Web-based approach.

These studies demonstrate that Web-based surveys are as effective or better than other modes in garnering survey responses and obtaining sound data. These findings largely address the fundamental concerns of maintaining data validity and obtaining sufficient numbers of responses raised when making the decision to migrate to a Web-based platform.

Online Surveys in Epidemiological Research

Despite results showing that Web-based questionnaires are as good or better than other survey modes, the field of epidemiological research has been slow to adopt Web-based methodology. A meta-analysis of epidemiology-related publications in seven high-impact general medical and epidemiological journals in 2008-2009 found that only 1% had used any form of Web-based data collection, while interviews were used in 28% and paper-based questionnaires in 29% (some used multiple formats) [45]. There is therefore potential for growth in the use of Web-based data collection tools for epidemiological purposes. The migration to Web-based questionnaires is likely to increase as a growing proportion of the population gains Web access. For example, World Bank data show that 83% of Australian and 46% of Chinese populations now use the Internet [54].

Online Surveys in ALS Research

There appears to be only one other Web-based epidemiological study of ALS [44]. In that study, ALS patients who had enrolled electronically with the US National ALS Registry were recruited via email. Inclusion criteria were a diagnosis of ALS confirmed by a physician, knowledge of English, residence in the United States for at least 10 years, and age 21 years or older. Exclusion criteria were having also been diagnosed with Parkinson disease, parkinsonism, Alzheimer disease, dementia, poliomyelitis, or post-polio syndrome or having a family member with ALS. From the 2232 emails sent, completed surveys were received from 256 respondents who fulfilled the eligibility criteria, an enrollment rate of 11.5%. Among the topics covered in the survey were lifetime occupational history, occupational exposures, residential history, hobbies, physical activity, and military history.

We have also been given permission to recruit ALS patients from the US National ALS Registry. It will be of interest to compare our enrollment rate with that of Malek et al [44] since we have fewer exclusion criteria. Also, because our questionnaire is anonymous we predict more people will feel comfortable supplying personal information about themselves.

Limitations of a Web-Based Questionnaire

Nonresponse Errors

A major concern in any survey is that the responses received are not representative of the population sampled (ie, nonresponse errors). It has been noted that the demographics of Internet users differ from the general population in that they tend to be younger and more educated [46,47]. However, one study that examined computer literacy and educational status among Web survey participants found that a substantial portion of their respondents considered themselves inexperienced in computer and Internet skills, and that those with less education were more accepting of the burden of completing an Internet survey [34].

A review of 11 Web-based surveys of people aged 65 years or older found that limitations for this age group were similar to those among all age groups [55]. One of the studies included in this review found that the mean age of Web-based participants (70 years) was lower than the age of face-to-face respondents (81 years) [56]. In our study, the average age of Web-based respondents (54 years) was slightly younger than that of paper-based respondents (60 years). This may imply some preference for the Web-based questionnaire among younger people, but direct comparisons between respondents in these two questionnaires are difficult to make. In our paper-based questionnaire, for example, all respondents also had to give a blood sample, which may have discouraged some younger people from participating.

Of note in our study, respondents are likely to be in the 40 to 70 year age group since this is the typical range for ALS. Therefore, age and educational status are unlikely to substantially limit participation in our Web-based questionnaire. We think that nonresponse error for our questionnaire will be minimal since most respondents are likely to have a strong interest in the subject.

Concerns About Safety of Personal Information

As with all uses of the Internet, there are concerns about safety and confidentiality of the data provided [45]. Our questionnaire largely circumvents this issue because all data are being

collected as anonymous responses, and our data are secured on password-protected servers and computers.

Inability to Get Further Information or DNA Samples From Respondents

Since we have no identifying details on our respondents, we cannot contact them individually to ask them further questions or to ask for DNA samples to look for gene-environment interactions. However, there are now a number of databases containing large numbers of DNA samples from ALS patients, and should our study find risk factors for ALS, the same factors could be sought from patients who have donated DNA to these registries.

Inability to Obtain Physician Confirmation of Diagnosis

Since the responses are anonymous, we cannot obtain physician confirmation of the diagnosis of ALS or classify the cases using El Escorial criteria [57]. This is unlikely to be a major limitation since most ALS patients are well aware of their diagnosis. For example, of 88 people who self-reported a diagnosis of ALS to the US National ALS Registry, a check of their physician reports identified only 5 (6%) who did not have ALS [44]. We think the accuracy of the self-reporting of ALS diagnosis by our respondents will be improved by requesting them to select which subtype of ALS they have and asking them to contact their physician if they do not know this.

Conclusions

The majority of epidemiological studies have been conducted using paper-based questionnaires, face-to-face interviews, or telephone surveys. The literature now shows that Web-based questionnaires offer many advantages over traditional methods with few drawbacks. Our experience creating an online questionnaire illustrates these advantages. Furthermore, our questionnaire is being translated into non-English languages and opened up to participation worldwide. We hope the data obtained from this project will accelerate our understanding of ALS and lead to the development of effective treatment options and preventative strategies.

Acknowledgments

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Conflicts of Interest

None declared.

Multimedia Appendix 1

The previous paper-based ALS risk factor questionnaire used by the Australian MND DNA Bank. A number of these questions were modified to fit the present online format.

[PDF File (Adobe PDF File), 75KB - resprot_v4i3e96_app1.pdf]

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Abbreviations

ALS: amyotrophic lateral sclerosis
ALS-FRS: ALS Functional Rating Scale
FTD: frontotemporal dementia
MND: motor neuron disease

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Chapter 3: Does the index to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)?

After completing the development of ALS Quest, the questionnaire was launched in January 2015. To enable ALS Quest to reach a worldwide audience, work had also commenced on obtaining translations of the questionnaire. This effort, as well as an overview of the implementation of the questionnaire, is summarized further in Appendix B. This information was presented at the annual meeting of the International Alliance of ALS/MND Associations in 2017 (Appendix C) and as a poster at the 28th International Symposium on ALS/MND in 2017 (Appendix D).

Once ALS Quest had been opened to participants, responses were collected quickly. Therefore, analysis of the data began within a relatively short time frame. Preliminary results from ALS Quest were presented in a poster at the MND Australia meeting in November 2015 (Appendix E). By late 2016, 800 responses had been collected so that sufficient data were available for further analyses.

One goal of ALS Quest was to assess novel risk factors that were either not published in the literature or had only been tested in a limited fashion. One such risk factor was prenatal testosterone, which can be evaluated by looking at the ratio of the index and ring fingers, the 2D:4D ratio. The lower this ratio, the higher the exposure to prenatal testosterone. This topic was chosen because it might provide insight into a link between physical activity and ALS. While physical activity has often been examined as a risk factor for ALS, the basis for such a link is unclear. It may not be athletic participation *per se* that leads to ALS, but rather that the link between athletics and ALS may be that both are related to higher prenatal exposure to testosterone, represented by a lower ratio of the 2D to 4D finger lengths [2-4].

One study had suggested such a link between prenatal testosterone and ALS based on finger lengths [2]. However, the numbers of participants were small and the control group was primarily composed of the female spouses of male ALS patients, which resulted in a lopsided gender balance (and finger ratios are influenced by gender). I was able, with the large number

of respondents available, to analyse male and female results separately, which also took into account the gender differences that have been described in ALS [1].

This analysis was conducted and published as the following paper in this chapter. The results showed that the group of respondents to ALS Quest had the same male-female difference in 2D:4D ratio noted in the literature, but that the 2D:4D ratios did not differ between ALS respondents and controls. These results were presented in a poster at the 28th International Symposium on ALS/MND in 2017 (Appendix F).

To complement this work, I conducted a validation study to evaluate how accurately people could measure their fingers, by comparing personal measurements with researcher measurements from photographs (Appendix G).

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Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case–control study

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ABSTRACT

Objectives The ratio of the length of the index finger (2D) to the ring finger (4D) (2D:4D) has been reported to be lower (ie, 2D<4D) in people with amyotrophic lateral sclerosis (ALS) than non-ALS controls. This has led to suggestions that exposure to increased prenatal testosterone, which also lowers this ratio, could be a risk factor for ALS. In an attempt to test this hypothesis, we examined 2D:4Ds from large numbers of patients with ALS and controls.

Setting An online multilingual questionnaire enabling respondents to measure their own index and ring finger lengths.

Participants Of the initial 949 respondents, 572 remained for analysis after elimination for inability to straighten fingers, not answering the question, statistical outliers and aged <40 years. Respondents remaining for analysis were 202 patients with ALS (125 males, 77 females) and 370 non-ALS controls (112 males, 258 females).

Results Unpaired t-tests with 95% CIs were used to assess differences in mean 2D:4Ds. Males had significantly lower mean 2D:4Ds than females, in both ALS and control groups, for both left and right hands. No significant differences were found in 2D:4Ds between ALS and control groups, in either males or females, for either left or right hands. Receiver operating characteristic curves showed no power for 2D:4Ds to predict ALS status in either males or females.

Conclusions 2D:4Ds did not differ between patients with ALS and controls in this study. This was despite the dataset being large enough to confirm the established finding of lower 2D:4Ds in males compared with females. These findings do not support the hypothesis that exposure to increased prenatal testosterone is a risk factor for ALS. A putative lower 2D:4D has been proposed to explain the link between ALS and exercise, but our results indicate that other exercise-related factors are more likely to explain this association.

Strengths and limitations of this study

- Use of an online questionnaire enabled recruitment of larger numbers of patients with amyotrophic lateral sclerosis (ALS) and controls than previously available for this type of study.
- The larger numbers of respondents allowed for statistically robust separate analyses of male and female length of the index finger (2D) to the ring finger (4D) ratios.
- The accuracy of self-measured finger lengths was validated by the finding of similar gender differences (2D<4D in males, 2D≥4D in females) to those reported in previous studies.
- This was an international study, with comparable national, ethnic and cultural backgrounds in ALS and non-ALS respondents.
- Although patients with ALS had to self-report their disease status, self-reporting of ALS compared with physician's assessments has been shown to be accurate in over 90% of participants.
- 10% of ALS patients and 2% of controls were unable to straighten their fingers to provide measurements.
- Numbers of respondents are too limited to investigate interethnic and internation 2D:4D differences.
- More random errors and larger SD are generally found in self-reported data.

INTRODUCTION

Gender differences in the ratio of the length of the index finger (2D) to the ring finger (4D) (2D:4D) have been studied for over a century, with a lower mean ratio (ie, 2D<4D) being repeatedly found in males compared with females,[1–3] though with considerable overlap. The smaller average male 2D:4D is considered to be due to increased amounts of intrauterine testosterone relative to oestrogen.[4 5] The reason for this appears to be that during development the fetal ring finger has more plentiful androgen and oestrogen receptors than the index finger and so grows longer in the presence of a relative excess of testosterone.[6 7]

The 2D:4D has been used as an index of prenatal hormone exposure in a variety of physiological and psychological conditions, including athletic ability and strength,[3 8–10] fertility,[4] various behaviours[11] and sexual orientation.[12] The ratio has also been reported to

be different in diseases where gender imbalances can be marked or in which prenatal influences are suspected, including breast cancer,[13] prostate cancer,[14] Alzheimer's disease[15] and multiple sclerosis.[16]

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a progressive neurodegenerative disease with a usual postdiagnosis survival time of 2–5 years.[17] A previous study reported a lower mean 2D:4D in patients with ALS compared with controls.[18] However, subject numbers in this study were too limited to detect the lower 2D:4D usually reported in males. To see if the results of this study could be validated with a larger number of subjects, we used data from an international online questionnaire[19] in which ALS and non-ALS respondents self-measured the lengths of their ring and index fingers.

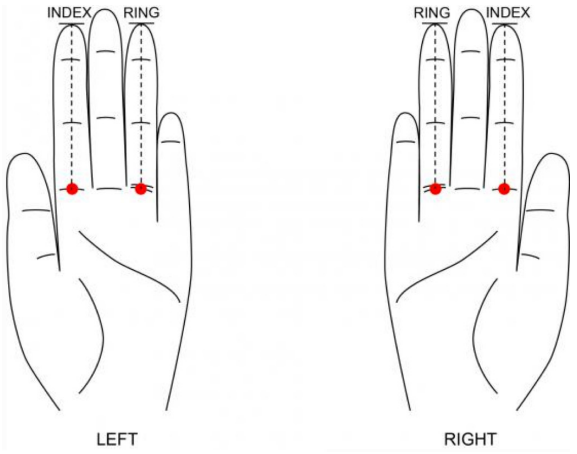

Figure 1 The diagram that appears in the online questionnaire to assist respondents to measure their finger lengths.

METHODS

Setting

This is a case–control study using data from an international, multilingual, web-based questionnaire ('ALS Quest') designed to look for risk factors for ALS. Details on the design and implementation of the questionnaire have been published.[19] The full questionnaire, which uses Qualtrics survey software, can be viewed at www.alsquest.org and completed online. A PDF version of the questionnaire is available for downloading as online supplementary file S1 .

Responses to the questionnaire collected between January 2015 and February 2017 were used for analysis. Respondents for the questionnaire were recruited via national and state ALS/MND associations, national ALS registries, the internet and social media. No personally identifying data were collected to allow the respondents to remain anonymous. The project was approved by the Human Ethics Committee of the Sydney Local Health District.

Cases were respondents who replied to a question about having been diagnosed as having ALS with 'Yes, I have been diagnosed with ALS/MND'. Controls were participants who answered this question with 'No, I have not been diagnosed with ALS/MND'. To maintain anonymity, information on disease status was not requested from attending physicians but was self-reported.

Finger measurements

The guide on how to perform finger measurements consisted of a diagram (figure 1) together with the following instructions: 'Please measure the lengths of your index (second) and ring (fourth) fingers on each hand. The diagram below shows you how to make the measurements. If you can only straighten out the fingers on one hand, only record the measurements for that hand. If possible, please take off any rings prior to measuring your fingers. First lay the back of your hand out flat on a level surface, with your fingers together. Then, with a rigid millimetre ruler (not a tape measure), measure from (1) the tip of the finger to (2) the middle of the crease at the bottom of the finger. The ring finger often has two of these creases, so here measure from the crease that is closest to the wrist'. Drop-down lists with numbers ranging from 35 to 135 mm allowed respondents to select the relevant finger lengths.

Other data analysed

Other questionnaire data used in the study (answers mostly selected from drop-down lists) were: date when consent was given to conduct the questionnaire, age, gender, country of residence, country of birth, ancestry, cultural group, dominant hand and family history of ALS. People with ALS were asked their date of diagnosis, subtype of ALS and to complete the ALS Functional Rating Scale (ALSFRS-R)[20] which for this study was scored in reverse so that higher scores indicated more advanced disease.

Statistical analyses

Data from the survey software were transferred to a Microsoft Excel file, where 2D:4Ds were calculated by dividing the length of the index finger by the length of the ring finger. IBM SPSS Statistics for Macintosh (V.22) and GraphPad Prism V.7 were used to create percentage frequency histograms, calculate unpaired t-tests with 95% CIs to compare means and perform Pearson's test for correlations. Receiver operating characteristic (ROC) curves assessed the sensitivity and specificity of 2D:4Ds to distinguish between ALS and controls. Effect sizes were calculated using G*Power.[21] Significance was assessed at the 0.05 level.

Exclusion criteria

Respondents were excluded from analysis (figure 2) if they: (1) could not straighten the fingers of both their hands, (2) did not answer the finger length question, (3) had extreme outlier results (identified on SPSS as being more than three times the IQR) and (4) were under the age of 40 years (to limit the number of control

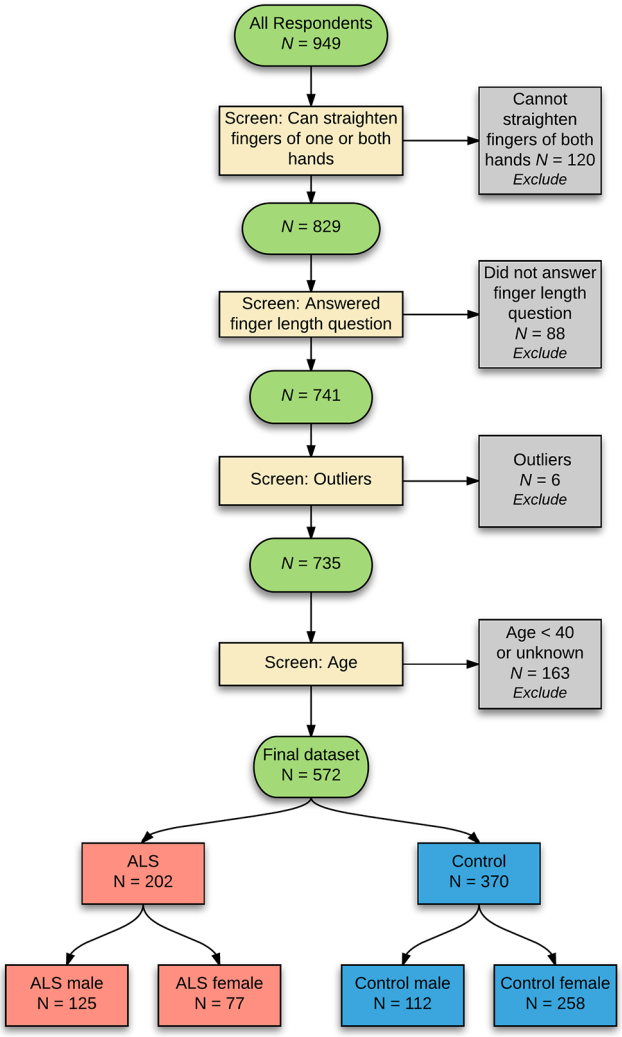

Figure 2 Flow chart of the selection of study respondents. ALS, amyotrophic lateral sclerosis.

respondents who might later be diagnosed with ALS) or did not answer the age question.

RESULTS

Cases and controls

Extreme outliers were five 2D:4D results and one left hand/right hand 2D:4D difference; when these were excluded, all self-measured values produced ratios ranging from 0.8 to 1.2, the range considered to be likely to reflect real values.[22] After all exclusion criteria were applied, the original number of 949 respondents was reduced to a final dataset number of 572 (figure 2). These remaining respondents comprised 202 ALS cases (125 male, 77 female) and 370 non-ALS controls (112 male, 258 female). The mean age of ALS patients was 61.7 years (SD 9.1 years, range 40–83 years) and of controls was 57.4 years (SD 10.4 years, range 40–86 years). Original finger length measurements from the analysed respondents (with genders and ages) are available as online supplementary file S2.

Sources of information about the questionnaire

The most common sources of information about the questionnaire cited by respondents were: ALS associations (36% of respondents), the internet (22%), friends (9%), patients with ALS (7%), health professionals (6%), community groups (5%), Facebook (4%), the USA CDC National ALS Registry (3%), the Canadian Neuromuscular Disease Registry (2%) and ALS researchers (2%).

Demographic and clinical characteristics

The composition of the ALS and control groups was similar with regards to country of birth, country of residence, ancestry and cultural group (table 1). The majority of respondents resided in Australia, the USA and Canada, though residents of a further 26 countries supplied responses. As reported by the ALS respondents, 52% had 'classic' (upper and lower motor neuron variant) ALS, 10% progressive bulbar palsy, 10% primary lateral sclerosis (upper motor variant), 7% progressive muscular atrophy (lower motor neuron variant), 9% 'other' and 9% did not know their subtype of ALS. Seven per cent of ALS patients had a relative with ALS and were classified as 'familial' ALS. Most controls (68%) reported no relatives with ALS, whereas 24% had one relative with ALS and 8% had more than one relative with ALS.

Comparison of 2D:4Ds between males and females

Left and right hand 2D:4Ds were significantly smaller in males compared with females, both in patients with ALS and controls (figure 3). The mean difference was slightly greater in patients with ALS than in controls (table 2).

Comparison of 2D:4Ds between patients with ALS and controls

Because of the mean 2D:4D differences found between our male and females, comparisons between ALS and control groups were undertaken in males and females separately. The frequency distributions of 2D:4Ds from both the left and right hands of ALS and control respondents were similar (figure 4). No significant differences in mean 2D:4Ds were seen between ALS and controls groups, in either the left or right hands, of either males or females (figure 3, table 2). Mean 2D:4Ds differed at only the third decimal place between these groups, and the 95% CIs spanned the zero difference in means (figure 3).

ROC curves of left and right hands showed that 2D:4Ds did not predict ALS status in either males or females (figure 5). The curves were close to the diagonal along the line of no-discrimination, with areas under the curve close to 0.5, indicating that the 2D:4Ds were neither sensitive nor specific to ALS status.

Rate of ALS progression

An estimate of the rate of ALS progression was obtained by dividing the reverse ALSFRS-R score by the number of months since diagnosis. The rate of ALS progression did not correlate significantly with 2D:4D, in either the left hand (r 0.072, p 0.317) or the right hand (r 0.082, p

Table 1 Demographic characteristics of respondents

ALS	n (%)	Control	n (%)
Country of birth			
USA	72 (36)	Australia	228 (62)
Australia	55 (27)	Other (<3% each)	65 (18)
Canada	32 (16)	USA	33 (9)
Other (<3% each)	22 (11)	UK	23 (6)
UK	11 (5)	South Africa	10 (3)
South Africa	5 (3)	Spain	10 (3)
Spain	5 (3)		
Country of residence			
USA	73 (36)	Australia	282 (76)
Australia	69 (34)	USA	39 (11)
Canada	38 (19)	Other* (<1% each)	27 (7)
Other* (<3% each)	12 (6)	Canada	5 (1)
South Africa	5 (3)	New Zealand	5 (1)
Spain	5 (3)		
Ancestry			
Other (<6% each)	82 (41)	Other (<4% each)	125 (34%)
Australian	34 (17)	Australian	105 (28)
English	28 (14)	English	66 (18)
American	16 (8)	Irish	35 (10)
Irish	16 (8)	British	20 (5)
Canadian	13 (6)	Scottish	15 (4)
German	13 (6)		
Cultural group			
Australian	57 (28)	Australian	238 (64)
American	43 (21)	Other (<2% each)	63 (17)
Other (<3% each)	43 (21)	American	24 (7)
Canadian	29 (14)	English	22 (6)
English	17 (8)	Spanish	10 (3)
German	6 (3)	Dutch	8 (2)

*Other countries of residence (in alphabetical order): Argentina, Belgium, Brazil, China, Colombia, Denmark, Ecuador, Egypt, Finland, Germany, Iran, Ireland, Italy, Japan, Lebanon, Luxembourg, Mexico, the Netherlands, Portugal, Russia, Slovakia, South Korea, Sweden, Switzerland, Turkey and UK.
Some subsets do not add up to the total number of respondents because of answers to particular questions not being given.
ALS, amyotrophic lateral sclerosis.

0.251), indicating the ratio had no influence on disease progression.

Sporadic and familial ALS

People with sporadic ALS (93% of cases) had no significant differences in 2D:4Ds to those with familial ALS (7%), for either the left or right hands, in either males or females. Similarly, no significant differences were seen in 2D:4Ds between non-ALS controls who had either no family member with ALS (68%), one family member with ALS (24%) or two or more family members with ALS (8%), for either hand in either gender.

Left–right symmetry of finger lengths and 2D:4Ds

No significant differences were found between the lengths of the left and right index fingers, or the left and right ring fingers, in either gender or either disease status group, indicating no asymmetry of left-right finger lengths in our respondents.

No significant differences between left and right hand 2D:4Ds of individual respondents were found, suggesting that respondents did not have difficulty measuring fingers of either their left or right hands. 2D:4Ds were also compared between male/female and ALS/control groups using dominant or non-dominant hands for

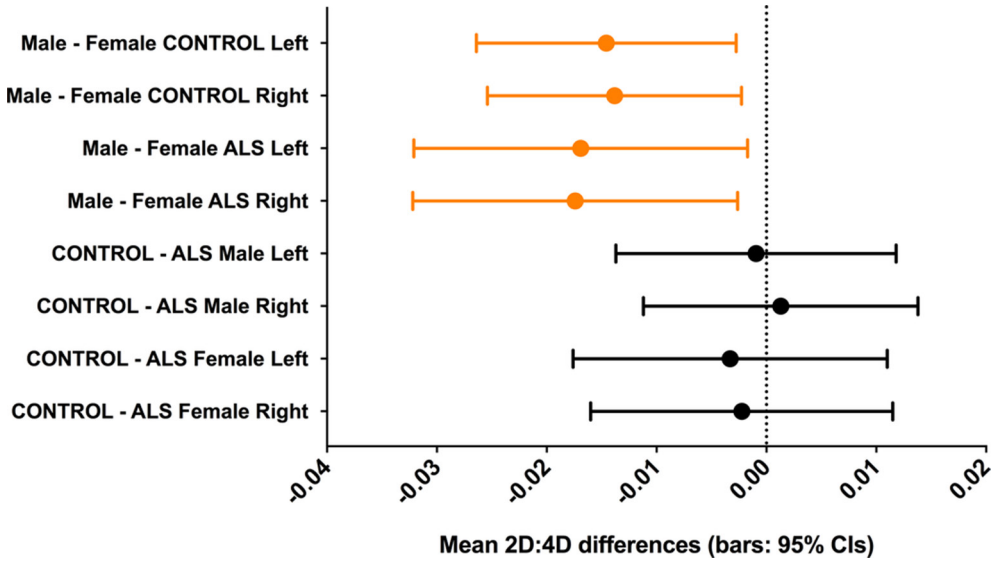

Figure 3 Differences in mean 2D:4Ds between male and females (upper segment, orange) and between patients with ALS and controls (lower segment, black). Mean 2D:4Ds (filled circles) are significantly smaller in males compared with females, in both control and ALS groups. No differences are seen in mean 2D:4Ds between control and ALS respondents, for either males or females, with the mean differences all close to zero, and the 95% CIs (bars) spanning the zero difference (dotted line). 2D:4D, ratio of the length of the index finger (2D) to the ring finger (4D); ALS, amyotrophic lateral sclerosis

measuring (85% of respondents were right-hand dominant, 10% left-hand dominant and 5% ambidextrous). The results of these analyses were the same as above (ie, males had lower 2D:4Ds, but no 2D:4D differences were found between patients with ALS and controls), indicating that use of the non-dominant hand to hold the ruler did not affect measurements.

Table 2 Differences in mean 2D:4D ratios (with p values and effect sizes) between males and females, and between patients with ALS and controls

Groups (n)	Mean 2D:4D (SD)	Mean 2D:4D difference (95% CI)	p Value	Effect size (d)
Male–female left hand				
Male control (112)	0.978 (0.0467)	−0.015 (-0.026 to −0.0028)	0.016	0.28
Female control (258)	0.993 (0.0557)			
Male ALS (124)	0.979 (0.0521)	−0.017 (-0.032 to −0.0017)	0.029	0.32
Female ALS (75)	0.996 (0.0537)			
Male–female right hand				
Male control (111)	0.979 (0.0461)	−0.014 (-0.025 to −0.0023)	0.019	0.28
Female control (258)	0.993 (0.0542)			
Male ALS (124)	0.978 (0.0508)	−0.017 (-0.032 to −0.0026)	0.021	0.34
Female ALS (77)	0.995 (0.0529)			
Control–ALS left hand				
Male control (112)	0.978 (0.047)	−0.00094 (-0.014 to 0.012)	0.884	
Male ALS (124)	0.979 (0.0521)			
Female control (258)	0.993 (0.0557)	−0.0033 (-0.018 to 0.011)	0.649	
Female ALS (75)	0.996 (0.0537)			
Control–ALS right hand				
Male control (111)	0.979 (0.0461)	0.0013 (-0.011 to 0.014)	0.835	
Male ALS (124)	0.978 (0.0508)			
Female control (258)	0.993 (0.0542)	−0.0022 (-0.016 to 0.012)	0.751	
Female ALS (77)	0.995 (0.0529)			

2D:4D, ratio of the length of the index finger (2D) to the ring finger (4D); ALS, amyotrophic lateral sclerosis.

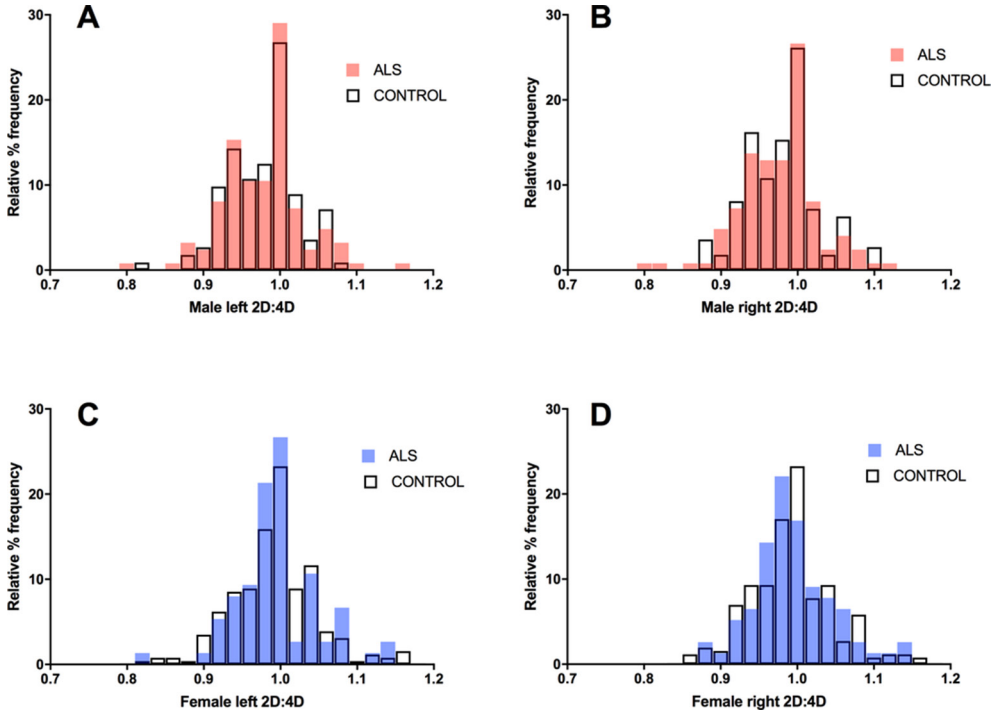

Figure 4 Overlapping histograms show the similar percentage frequency distributions of 2D:4Ds between ALS and control respondents, for both males (A: left hand, B: right hand) and females (C: left hand, D: right hand). 2D:4D, ratio of the length of the index finger (2D) to the ring finger (4D); ALS, amyotrophic lateral sclerosis.

DISCUSSION

We found no differences in mean 2D:4Ds between ALS and non-ALS respondents, with males and females being analysed separately, and 2D:4Ds had no ability to predict ALS status. This was despite our dataset being large enough to confirm the established lower mean 2D:4D found in males compared with females.

Internet surveys are able to gather data from large numbers of participants, but concerns have been raised about superficial answering of questions and multiple responses from individuals.[23] These would be unlikely in the present survey, with most respondents either having the serious condition of ALS or having knowledge about the disease. Furthermore, the ALS Quest survey is detailed and extensive, taking most respondents about 2 hours to complete, often in multiple sessions, so respondents who complete the survey are likely to be motivated to give accurate data. Multiple answers are also not likely since the questionnaire is set up to allow only one response per internet browser/computer.

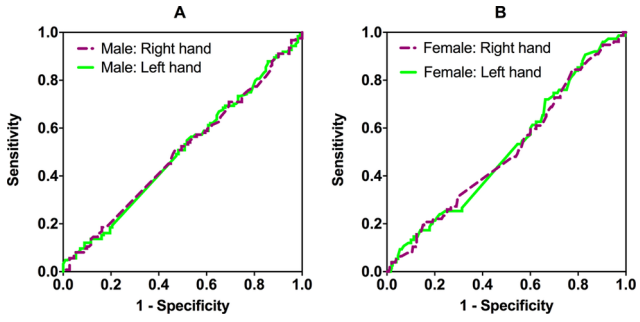

Figure 5 Receiver operating characteristic curves of 2D:4Ds in both males (**A**) and females (**B**) are close to the diagonal line of no-discrimination (not drawn since it would obscure the curves). Areas under the curve are close to 0.5, indicating that the 2D:4Ds are neither sensitive nor specific to ALS status. 2D:4D, ratio of the length of the index finger (2D) to the ring finger (4D); ALS, amyotrophic lateral sclerosis.

An important issue concerns the accuracy of the self-measured finger lengths in this study. Comparisons between large numbers of Internet and conventionally collected survey data have shown consistency with researcher-measured samples.[23] Web-based 2D:4D measurements appear to add some random noise to the data, but do not lead to systemic errors.[24] When self-reported finger measurements were compared with those measured on photocopies by researchers, the self-reported lengths were found to be valid for the study of 2D:4D, provided outliers were removed and large sample numbers were available.[25] These recommendations were followed in the present study: after a few extreme outliers (only 1% of the total) were removed, our self-reported 2D:4Ds produced ratios ranging from 0.8 to 1.2. Values within this range are considered to represent real values, as reported in another online 2D:4D study.[22] Nevertheless, the 2D:4D ratios in our study have larger SD than those in an experimenter-measured finger length study.[26] Our calculated 2D:4Ds therefore have relatively high levels of random error, with weakening of any relationships between 2D:4D and ALS. This can also be seen from our gender difference findings, which while significant and being close to those previously reported,[27] had smaller effect sizes, with a reduction from d values of

0.5–0.6 in experimenter-measured studies[26] to 0.25–0.35 in our online study. This emphasises the need for large numbers of subjects in web-based 2D:4D studies.

Ideally, to be able to confidently exclude small between-group differences in 2D:4D, future studies in ALS would be of about 200 male and female ALS and control subjects (ie, 800 subjects in all). In addition to direct experimenter measurements of finger lengths, it would be useful to photograph the hands under consistent conditions (eg, image size, lighting, camera–finger distance) and use a computer-based measurement tool on the images to assess inter-rater and test–retest reliability. However, undertaking such a large study in a single clinical setting would be difficult. As a rule of thumb, any study that is insufficiently powered to detect the known gender differences in 2D:4D is unlikely to be able to detect biologically significant 2D:4D differences between disease and control groups.

Other limitations of our study are: (1) in order for respondents to remain anonymous they had to self-report their ALS status. We could not therefore classify their types of ALS using El Escorial criteria or more recently suggested classification methods.[28] However, self-reporting of ALS status has previously been found to be 94% accurate when compared with physicians' responses.[29] Indirect confirmation of an accurate ALS diagnosis is suggested by 88% of ALS respondents being able to choose their subtype of ALS via a multiple choice question (patients were encouraged to contact their physician if they were unsure of their subtype) and by 98% of ALS respondents completing the ALSFRS-R, which when completed online has been shown to compare well with clinical evaluation. (2) The results may be biased towards selecting people with early ALS, since people with advanced ALS are those who would be most likely to be unable to straighten their fingers for measurements. However, there is no evidence that the pathogenesis of ALS differs between the early and later stage of the disease, so this should not affect our conclusions. (3) Some people with ALS have cognitive deficits[30] and so may not have been able to measure their fingers accurately. However, it is unlikely that anybody with a clinically significant cognitive deficit would have been able to complete the extensive online questionnaire.

Our results do not corroborate the findings of a previous study that suggested the 2D:4D is lower in patients with ALS than in controls.[18] This study had modest numbers of participants, with 33 ALS males (70%) and 14 ALS females (30%), compared with 20 control males (32%) and 43 control females (68%). The combination of an excess of males (with gender-related 2D<4D) in the ALS group and an excess of females (with gender-related 2D≥4D) in the control group could have led to a finding of a decreased mean 2D:4D in the ALS group, though raw results were not available to test this. The authors of this study acknowledge that insufficient numbers were available to look for 2D:4D gender differences.[18] Our failure to replicate the results of this study are perhaps not surprising, given that it is androgen deficiency that affects motoneurons adversely,[31 32] so androgen excess during development (which causes a low 2D:4D) would not a priori be expected to be a risk factor for later motoneuron loss.

A lower 2D:4D ratio is found in people who have athletic ability.[3 33] Based on the previous report of a lower 2D:4D in ALS patients,[18] it was hypothesised that the suspected association of physical activity with ALS is because people with ALS have a prenatal disposition to both ALS and athleticism due to exposure to higher prenatal testosterone, rather than the physical activity itself being responsible for the increased ALS risk.[18 34] Our finding of no lower 2D:4D in ALS makes this hypothesis unlikely. Moreover, the evidence for physical activity as a risk factor for ALS has recently been questioned.[35] However, if further studies confirm this ALS–exercise link, other reasons for physical activity being associated with ALS need to be considered, such as those listed for football (soccer) players who have an increased risk of ALS, which include the use of toxic substances to improve performance, exposure to environmental toxins such as fertilisers or herbicides on playing fields or sport-related trauma.[36] A further suggestion that has been put forward to explain an ALS–exercise link is that exercise increases transmission of circulating toxicants across the neuromuscular junction into motor axons, from where the toxicants travel to the motoneuron cell body by retrograde axonal transport and trigger the disease.[37]

Three future 2D:4D studies could be undertaken once more responses to international questionnaires such as ours are available. (1) A study using self-measured finger lengths has shown that the 2D:4D varies with ethnicity[38] (this would not have affected our results since our respondents were mostly of European origin). In addition, 2D:4Ds may be different within gene pools from people of the same ethnicity.[39] Examination of ALS-related 2D:4D differences in various ethnic groups and between different nations will be possible once more respondents from other ethnic groups and countries provide relevant finger length data. (2) The 2D:4D appears to be lower in people born in late autumn, possibly because of the effects of melatonin on fetal testosterone.[40] This time of season overlaps with the same period (late summer to early winter) in which ALS birth rates have been reported to be increased.[41] A future study on season/ALS/2D:4D interactions will be possible once sufficient data on these variables are collected. (3) Between-nation gender differences in 2D:4D have been described,[42] so it would be of interest to gather sufficient numbers of ALS and control responses from different countries to be able to compare 2D:4Ds between these countries.

In conclusion, our online case–control study found no differences between the 2D:4Ds of ALS and non-ALS respondents. The study does not therefore support the hypothesis that exposure to prenatal testosterone is a risk factor for ALS. If physical activity is confirmed to be a risk factor for ALS, reasons other than a low 2D:4D need to be sought to explain this association. Future studies

of ethnic, time-of-birth and multination subgroups may detect more nuanced relationships between ALS and 2D:4D when data from larger numbers of ALS and non-ALS respondents become available.

Acknowledgements We thank all respondents who took the time to complete the detailed ALS Quest questionnaire, and volunteers who helped translate the questionnaire into different languages. Respondents were alerted to the availability of the questionnaire by many worldwide national and state-based ALS associations, the International Alliance of ALS/MND Associations, the USA CDC National ALS Registry, the Canadian Neuromuscular Disease Registry and community clubs such as those run by Rotary, Lions, the Returned and Services League and the Men's Shed Association. Lucidchart software was used to create the flow diagram.

Contributors JAPK and RP planned the project, acquired and analysed the data, wrote the manuscript (JAPK wrote the first draft), approved the final version submitted and are accountable for all aspects of the work.

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Competing interests The study was partially funded by the Aimee Stacy Memorial Bequest and the Ignacy Burnett Motor Neuron Disease Bequest, both managed by the University of Sydney.

Patient consent Participants consented to answering the online questionnaire by following these instructions: "Please click on the I Consent button below to indicate your consent to enter data into the questionnaire. By clicking this button, I: 1. Acknowledge that I have read the Information for Participants above and agree to participate in this research. 2. Understand that I will not be asked for any personal information that could identify me, so the study is anonymous and strictly confidential. 3. Freely choose to participate in the study and understand that I can withdraw my questionnaire answers at any time until I click the Submit button at the end of the questionnaire."

Ethics approval The Human Ethics Committee of the Sydney Local Health District.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional unpublished data from the study (apart from the two supplementary files) are available.

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Chapter 4: Are people with amyotrophic lateral sclerosis (ALS) particularly nice? An online international study of the Big Five personality factors

An aspect of ALS that had been more in the realm of anecdote than scientifically studied is that ALS patients are generally considered "nice." However, this had until now only been qualitatively assessed [1].

The ALS Quest questionnaire included a personality inventory that could provide insight into the personality types of ALS patients. A comparison of the personalities of controls and ALS respondents, which were evaluated separately by gender, found that male ALS respondents had higher scores than male controls for Conscientiousness and Extraversion. Female ALS respondents had higher scores than female controls for Agreeableness, Conscientiousness, and Extraversion, and a lower score for Neuroticism.

These findings, which are presented as a publication in this chapter, indicate that there are personality differences between ALS and control respondents. These differences could arise as a result of germline genetic variants, since personality can be considered to result partially from genetic factors [2].

This study of personality of people with ALS also raises the possibility that personality contributes to some of the purported risk factors for ALS – for example, if people who get ALS are more extroverted, they may be more likely to smoke [3].

These results were presented in a poster at the 28th International Symposium on ALS/MND in 2017 (Appendix H).

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ORIGINAL RESEARCH

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Are people with amyotrophic lateral sclerosis (ALS) particularly nice? An international online case–control study of the Big Five personality factors

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Abstract

Background: Many people with ALS have been suggested to have a "nice" personality, but most ALS personality studies to date have had limited numbers of participants and have not taken into account personality differences between genders. We used Big Five Inventory data obtained from an online questionnaire looking for risk factors for ALS to investigate personality traits in large numbers of people with ALS and controls.

Methods: A total of 741 questionnaire respondents aged 40 years and over indicated the extent to which they agreed with each of the 44 Big Five Inventory statements. Respondents were 339 with ALS (212 male, 127 female) who responded to the statements as they applied to them before their diagnosis and 402 controls (120 male, 282 female). Unpaired *t* tests with 95% confidence intervals were used to compare mean values of Big Five-factor scores.

Results: Female respondents taken together had higher mean scores for Agreeableness and Neuroticism than all male respondents. Male ALS respondents had higher mean scores than male controls for Conscientiousness and Extraversion. Female ALS respondents had higher mean scores than female controls for Agreeableness, Conscientiousness, and Extraversion, and a lower score for Neuroticism.

Conclusions: Many people with ALS have personality traits that are likely to underlie the perception they are particularly "nice." This raises the possibility that genetic polymorphisms that influence personality could play a role in ALS. Furthermore, different personality traits could underlie lifestyle choices that are currently thought to be risk factors for ALS.

KEYWORDS

amyotrophic lateral sclerosis, Big Five Inventory, case-control study, international, online questionnaire, personality, risk factors

1 | INTRODUCTION

People with amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, are often described by clinicians as having a particularly pleasant personality, which could be interpreted as "niceness" (Mehl, Jordan, & Zierz, 2017). Interest has persisted in this subject because of the possibility that a characteristic personality profile for ALS could give clues as to the underlying pathogenesis of the disease (Brown & Mueller, 1970; Grossman, Levin, & Bradley, 2006; Mehl et al., 2017).

The previous studies of personality differences in ALS go back to 1947 when ALS patients were reported to have a "cheerful" attitude (Veit, 1947). In 1970, a range of psychological tests characterized 10 ALS patients as having "active mastery" and "denial of affect," with the conclusion that an "association of ALS with a characteristic personality style, if confirmed, might have etiologic and prognostic implications" (Brown & Mueller, 1970). In 1977, a study of 40 ALS patients, using a variety of psychological tests, was unable to find any of the denial of affect (Houpt, Gould, & Norris, 1977) that had been reported in the 1970 study. It was suggested that small numbers, and issues with patient selection, were the reasons for this discrepancy. The next ALS personality study in 1978 found no differences in Minnesota Multiphasic Personality Inventory profiles of 38 ALS patients compared with a large number of medical patients without serious conditions (Peters, Swenson, & Mulder, 1978).

A different approach was taken in 2006 when 49 caregivers were asked to rate the premorbid personality traits of ALS patients, compared to non-ALS patients with chronic progressive conditions, using the NEO Five-Factor Personality Inventory (Grossman et al., 2006). ALS patients were rated as having comparatively lower openness, but the other factors of neuroticism, extraversion, agreeableness, and conscientiousness did not differ between the two groups.

When 36 physicians assessed the personality of their ALS patients in a 2017 study, using a shortened version of the NEO Five-Factor Personality Inventory, agreeableness was rated more highly than that reported by physicians caring for patients with non-ALS illnesses (Mehl et al., 2017). This supported the anecdotal description of people with ALS as being "nice."

In summary, ALS premorbid personality studies have to date given mixed results. No systematic studies of premorbid personality in large numbers of people with ALS have been reported, and none has taken into account gender differences in personality. In an attempt to find out if certain personality types are more common in people with ALS, including those associated

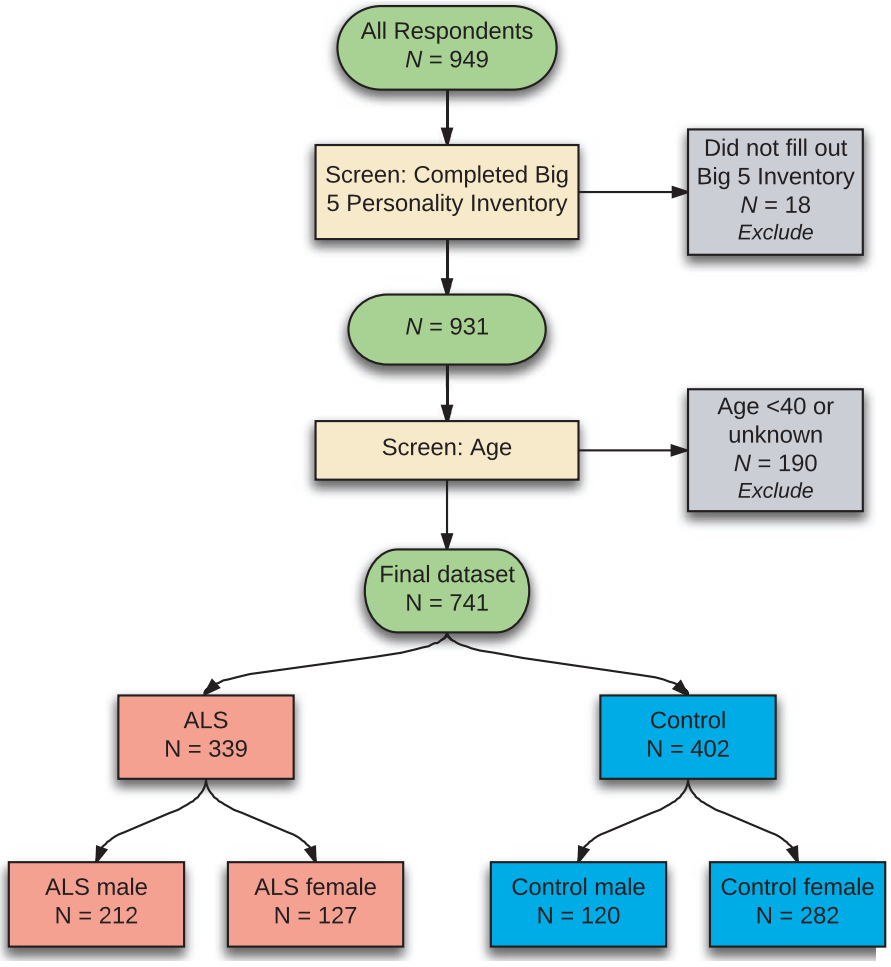

FIGURE 1 Selection of individuals for analysis. The flowchart shows the final dataset of 741 respondents after exclusion criteria were applied and final numbers of ALS and control individuals

with "niceness," we used personality data generated by the Big Five Inventory (John, Naumann, & Soto, 2008) from ALS Quest, an online international questionnaire designed to study risk factors for ALS (Parkin Kullmann, Hayes, Wang, & Pamphlett, 2015). The large numbers of ALS and control respondents to this questionnaire enabled the study of personality factors in men and women separately.

2 | METHODS

2.1 | Setting

This case–control study used data collected between January 2015 and February 2017 from a multilingual web-based questionnaire, ALS Quest (www.alsquest.org) (Parkin Kullmann & Pamphlett, 2017; Parkin Kullmann et al., 2015). Respondents were recruited via worldwide national and state ALS Associations, national ALS registries, the Internet, and social media. No personally identifying data were collected so respondents remained anonymous. Information on disease status was self-reported. Cases were respondents who stated "Yes, I have been diagnosed with ALS/MND." Controls were participants who stated "No, I have not been diagnosed with ALS/MND."

2.2 | Ethics, consent, and permissions

The project was approved by the Human Ethics Committee of the Sydney Local Health District. Participants consented to submit their questionnaire responses by clicking an "I consent" button after acknowledging that they had read the preceding Information for Participants section of the questionnaire and agreeing that they "Understand that I will not be asked for any personal information that could identify me, so the study is anonymous and strictly confidential" and "Freely choose to participate in the study and understand that I can withdraw my questionnaire answers at any time until I click the Submit button at the end of the questionnaire."

TABLE 1 Demographic characteristics of respondents

	ALS	*n* (%)	CONTROL	*n* (%)
Country of birth	United States	128 (38)	Australia	248 (62)
	Australia	83 (25)	Other (<2% each)	67 (17)
	Canada	48 (14)	United States	38 (10)
	Other (<1% each)	52 (15)	United Kingdom	24 (6)
	United Kingdom	17 (5)	Spain	13 (3)
	Spain	10 (3)	South Africa	10 (3)
Country of residence	United States	136 (40)	Australia	305 (76)
	Australia	103 (34)	United States	44 (11)
	Canada	56 (17)	Other* (<1% each)	28 (7)
	Other* (<1% each)	34 (10)	Spain	13 (3)
	Spain	9 (3)	Canada	5 (1)
			New Zealand	5 (1)
Ancestry	Other (<5% each)	162 (49)	Other (<4% each)	138 (35)
	Australian	51 (15)	Australian	115 (29)
	English	51 (15)	English	69 (17)
	American	27 (8)	Irish	37 (9)
	Irish	21 (6)	British	22 (6)
	German	21 (6)	Scottish	16 (4)
Cultural group	Australian	87 (27)	Australian	255 (64)
	American	81 (25)	Other (<2% each)	65 (16)
	Other (<2% each)	79 (24)	American	29 (7)
	Canadian	39 (12)	English	27 (7)
	English	28 (9)	Spanish	13 (3)
	Spanish	10 (3)	Dutch	8 (2)

Note. Other* countries of residence: Argentina, Belgium, Brazil, China, Colombia, Denmark, Ecuador, Egypt, Finland, Germany, Iran, Ireland, Italy, Luxembourg, Mexico, Netherlands, Portugal, Russia, Slovakia, South Africa, South Korea, Sweden, Switzerland, Turkey, and the United Kingdom.
ALS: amyotrophic lateral sclerosis.

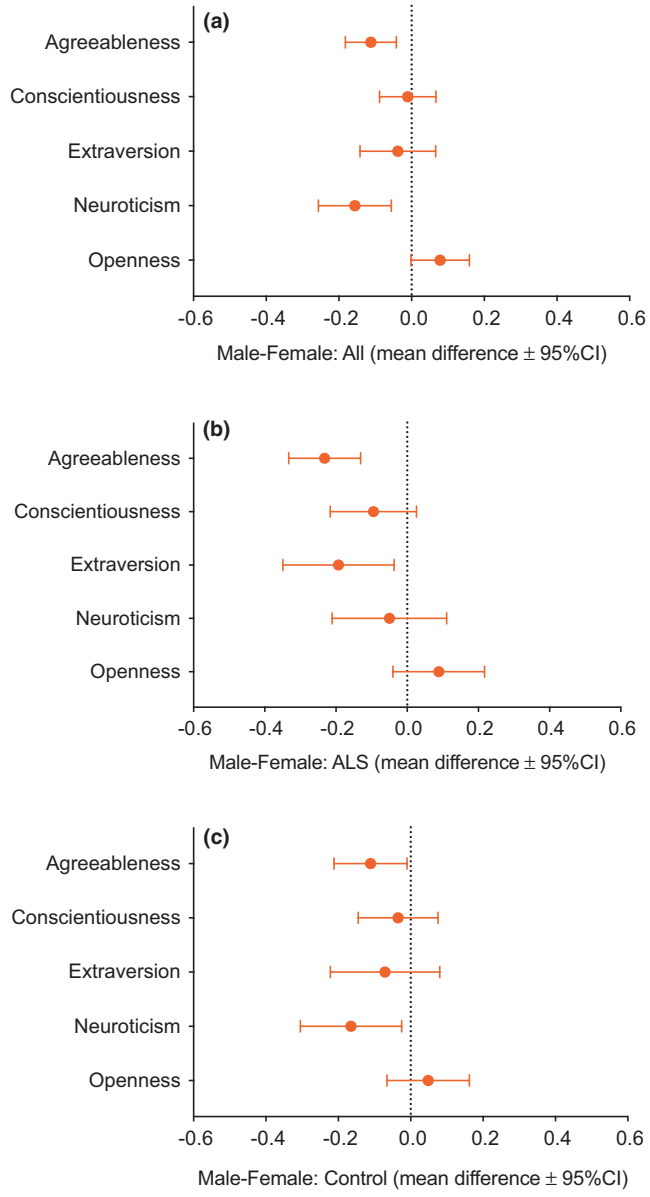

FIGURE 2 Mean differences (filled circles) and 95% confidence intervals (bars) in Big Five factors in males and females. Graphs for (a) males and female combined, (b) ALS males and females, and (c) control males and females show the differences in mean scores between groups. For example, in (a), it can be seen that women overall scored significantly more (i.e., the 95% CI bars do not cross the dotted zero line) for Agreeableness than men, while men tended to score more for Openness

2.3 | The Big Five Inventory

The Big Five Inventory provides scores for the personality factors of Agreeableness, Conscientiousness, Extraversion, Neuroticism, and Openness (John et al., 2008). These are calculated from a set of 44 statements about personality. Each statement is rated on a five-point Likert scale ranging from strongly disagree to strongly agree. Descriptors for these factors by the Big Five Inventory developers are as follows: (a) Agreeableness: altruism, tender mindedness, trust, and modesty; (b) Conscientiousness: thinking before acting, delaying gratification, following norms and rules, and planning, organizing, and prioritizing tasks; (c) Extraversion: sociability, activity, assertiveness, and positive emotionality; (d) Neuroticism: feeling anxious, nervous, sad, and tense; and (e) Openness: the breadth, depth, originality, and complexity of a person's mental and experiential life. The Big Five Inventory statements were translated into the language used by the respondent to complete the questionnaire. People with ALS were asked to provide responses they would have given to the statements *before* their diagnosis of ALS was made.

2.4 | Exclusion criteria

Respondents were excluded from analysis (Figure 1) if they did not complete the Big Five Inventory, they did not supply their age, or they were under the age of 40 years. The latter exclusion was to limit the number of control respondents who might later develop ALS and to avoid differences in personality between younger and older individuals (Srivastava, John, Gosling, & Potter, 2003).

2.5 | Big five-factor scores

A mean score, ranging from 1 to 5, was calculated for each factor, with higher scores indicating a greater expression of the factor. Percentage frequency histograms were created to compare the distributions of scores between ALS respondents and controls.

2.6 | Clinical characteristics

People with ALS were asked to complete the online version of the ALS Functional Rating Scale-Revised that has been designed for self-assessment (Maier et al., 2012; Parkin Kullmann et al., 2015) to assess their physical state at the time of taking the questionnaire; scores were inverted from the standard scale so that the higher the score the more impaired the function, with 0 indicating no impairment and 48 indicating very severe impairment. This online self-assessment of ALS severity has been shown to have excellent agreement with face-to-face application of the rating scale (Maier et al., 2012). The disease duration at the time of completing the questionnaire was calculated by subtracting the year of disease onset from the year of consenting to complete the questionnaire.

2.7 | Data analysis

Data from the Qualtrics server were transferred to GraphPad Prism 7 files. *F* tests were used to compare variances between groups, before unpaired *t* tests with 95% confidence intervals were used to compare mean values of Big Five scores. In only two groups (ALS male vs. female for Agreeableness and female ALS vs. control for Openness) were slight differences in variances found, but when these analyses were repeated using nonparametric Mann–Whitney tests, the same

TABLE 2 Differences in Big Five-factor scores between male (control *n* = 120, ALS *n* = 212) and female (control *n* = 282, ALS *n* = 127) respondents

Big Five factors	Group	Big Five-factor score mean (*SD*)	Big Five-factor score mean difference (95% CI)	*p* Value	Effect size (*d*)
1. Agreeableness	Male control	3.71 (0.51)	−0.11 (−0.21 to −0.01)	0.03	0.23
	Female control	3.83 (0.46)			
	Male ALS	3.83 (0.51)	−0.23 (−0.34 to −0.13)	<0.001	0.49
	Female ALS	4.06 (0.43)			
2. Conscientiousness	Male control	3.84 (0.52)	−0.04 (−0.15 to 0.08)	0.54	
	Female control	3.88 (0.51)			
	Male ALS	3.97 (0.56)	−0.10 (−0.22 to 0.03)	0.12	
	Female ALS	4.07 (0.53)			
3. Extraversion	Male control	3.10 (0.70)	−0.07 (−0.22 to 0.08)	0.36	
	Female control	3.17 (0.71)			
	Male ALS	3.32 (0.70)	−0.19 (−0.35 to −0.04)	0.02	0.27
	Female ALS	3.51 (0.71)			
4. Neuroticism	Male control	2.60 (0.63)	−0.17 (−0.31 to −0.03)	0.02	0.26
	Female control	2.77 (0.64)			
	Male ALS	2.52 (0.75)	−0.05 (−0.21 to 0.11)	0.54	
	Female ALS	2.57 (0.69)			
5. Openness	Male control	3.52 (0.54)	0.05 (−0.07 to 0.16)	0.40	
	Female control	3.47 (0.53)			
	Male ALS	3.57 (0.56)	0.09 (−0.04 to 0.22)	0.18	
	Female ALS	3.48 (0.62)			

Note. ALS: amyotrophic lateral sclerosis; *SD*: standard deviation; CI: confidence interval.

results were found. Correlations between the personality scores and the ALSFRS-R inverted scale and disease duration were measured with Spearman nonparametric r coefficients. Effect sizes (*d*) were calculated using G*Power software. Significance was assessed at the 0.05 level.

Lucidchart software was used to create Figures 1 and 7.

3 | RESULTS

3.1 | Cases and controls

A total of 741 respondents were eligible for analysis after exclusion criteria were applied (Figure 1). These comprised 339 ALS respondents (212 male and 127 female) and 402 non-ALS controls (120 male and 282 female). The mean age of ALS respondents was 61.3 years (*SD*: 9.3 years, range: 40–84 years) and of controls was 57.3 years (*SD*: 10.6 years, range: 40–89 years). Information on countries of birth and residence, ancestries, and cultural groups of respondents can be viewed in Table 1. The composition of the ALS and control groups was similar with regard to country of birth, country of residence, ancestry, and cultural group. The majority of respondents resided in Australia, the USA, and Canada, though residents of a further 27 countries supplied responses.

Sources of information about the questionnaire cited by respondents were as follows: ALS Associations (36%), the Internet (22%), friends (9%), people with ALS (7%), health professionals (6%), community groups (5%), Facebook (4%), the USA CDC National ALS Registry (3%), the Canadian Neuromuscular Disease Registry (2%), and ALS researchers (2%).

About 9% of ALS respondents had at least one relative who had been diagnosed with ALS and were classified as having familial ALS. The other 91% of ALS respondents were classified as having sporadic (or isolated) ALS. As reported by the ALS respondents, 58% had "classic" (upper and lower motor neuron variant) ALS, 9% progressive muscular atrophy (lower motor neuron variant), 8% progressive bulbar palsy, 8% primary lateral sclerosis (upper motor variant), 7% "other," 2% ALS/FTD (frontotemporal dementia), and 8% did not know their subtype of ALS.

When control participants were asked what their connection with ALS was, responses were (blood and nonblood) relatives (45%), spouses (10%), or friends (13%) of people with ALS, individuals from community, research, or medical professional groups (9%), or no specific (or another type of) connection to ALS/MND (23%). When controls were asked to identify what blood relatives with ALS they had, 58% reported no blood relatives with ALS, whereas 29% had one, and 13% more than one, blood relative/s with ALS.

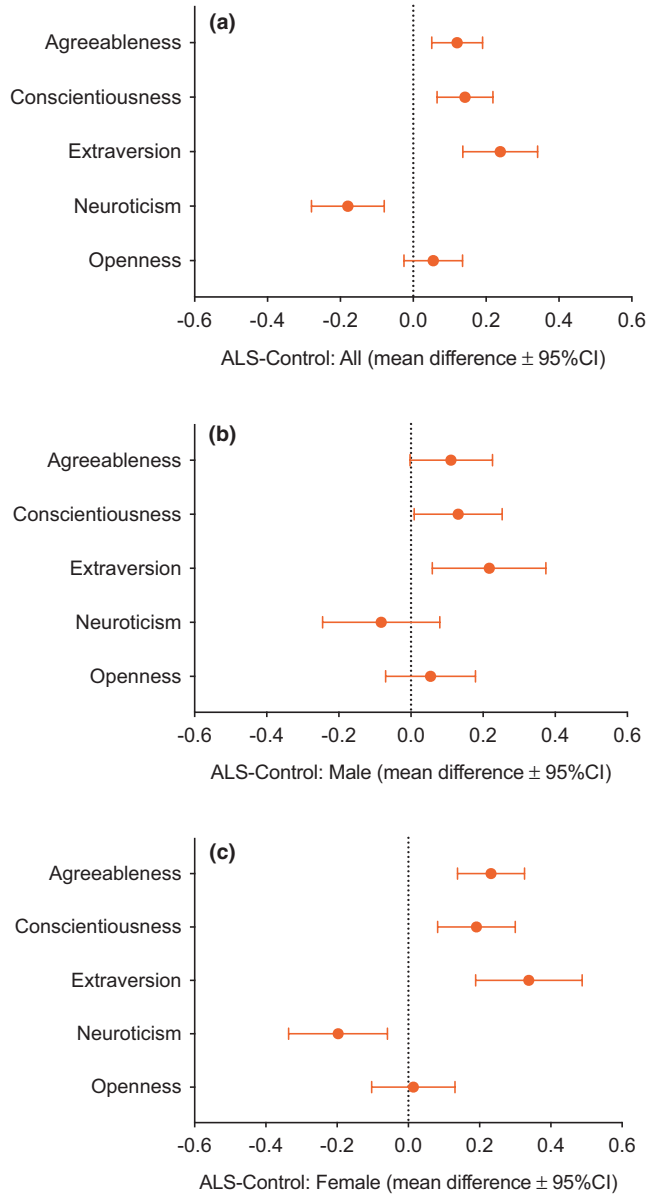

FIGURE 3 Mean differences (filled circles) and 95% confidence intervals (bars) in Big Five factors in ALS respondents and controls. Graphs for (a) males and female combined, (b) ALS males and controls, and (c) ALS females and controls show differences in mean scores. For example, in (a), it can be seen that ALS respondents overall scored significantly more for Agreeableness, Conscientiousness, and Extraversion, less for Neuroticism, and similarly for Openness

3.2 | Big Five-factor mean scores

3.2.1 | Male versus female differences

When all men and all women were compared, women scored higher than men for Agreeableness and Neuroticism (Figure 2a). No significant differences were seen between men and women for Extraversion, Conscientiousness, or Openness.

ALS females scored higher than ALS males for Agreeableness (effect size $d = 0.49$) and Extraversion ($d = 0.27$) (Figure 2b, Table 2). No significant differences were found between these two groups for Conscientiousness, Openness, or Neuroticism.

Control females scored higher than control males for Agreeableness ($d = 0.23$) and Neuroticism ($d = 0.26$) (Figure 2c, Table 2). No significant differences were found between these two groups for Conscientiousness, Openness, or Extraversion.

3.2.2 | ALS versus control differences

When all ALS respondents were compared to all controls, ALS respondents scored higher than controls for Agreeableness, Conscientiousness, and Extraversion, and lower than controls for Neuroticism (Figure 3a, Table 3). No significant difference was seen in Openness.

Male ALS respondents scored higher than male controls for Extraversion ($d = 0.31$) and Conscientiousness ($d = 0.24$) (Figure 3b, Table 3). No significant differences were found for Openness, Neuroticism, or Agreeableness.

Female ALS respondents scored higher than female controls for Agreeableness ($d = 0.54$), Conscientiousness ($d = 0.24$), and Extraversion ($d = 0.48$), and lower than controls for Neuroticism ($d = 0.30$) (Figure 3c, Table 3). No significant differences were found for Openness.

No significant differences in mean scores were seen when the 91% of sporadic ALS respondents were compared with the 9% of familial ALS respondents (data not shown).

3.3 | Correlation of Big Five-factor scores with ALS functional state and duration

Most ALS respondents had only mild or moderate loss of function as measured by the ALSFRS-R inverted score (Figure 4a); the median ALSFRS-R inverted score was 14. Most responded within the first four years after their ALS disease onset, with a long tail containing a few long-term survivors (Figure 4b); the median disease duration was 3 years. Correlation coefficients between the personality factors of Agreeableness, Conscientiousness, Extraversion, Neuroticism, Openness, and the ALSFRS-S inverted scores were low (respectively, $r = -0.03$, 0.06, 0.02, 0.02, and 0.08, all nonsignificant), with similar low personality score correlations with disease duration (respectively, $r = 0.06$, 0.00, 0.10, 0.01, and 0.03, all nonsignificant). The ALSFRS-R inverted score correlated significantly with disease duration ($r = 0.31$, $p < 0.001$).

3.4 | *Big Five-factor frequency distributions: ALS* versus *controls*

Frequency percentage histograms showed overlap in Big Five scores between ALS respondents and controls, both for men (Figure 5) and women (Figure 6), but with shifts in some ALS distributions to the right or left in line with the mean score results.

TABLE 3 Differences in Big Five-factor scores between ALS (male *n* = 212, female *n* = 127) and control (male *n* = 120, female *n* = 282) respondents

Big Five factors	Group	Big Five-factor score mean (*SD*)	Big Five-factor score mean difference (95% CI)	*p* Value	Effect size (*d*)
1. Agreeableness	Male control	3.71 (0.50)	−0.11 (−0.23 to 0.002)	0.06	
	Male ALS	3.83 (0.51)			
	Female control	3.83 (0.46)	−0.23 (−0.33 to −0.14)	<0.001	0.54
	Female ALS	4.06 (0.43)			
2. Conscientiousness	Male control	3.84 (0.52)	−0.13 (−0.25 to −0.009)	0.04	0.24
	Male ALS	3.97 (0.56)			
	Female control	3.88 (0.51)	−0.19 (−0.30 to −0.08)	0.001	0.38
	Female ALS	4.07 (0.53)			
3. Extraversion	Male control	3.10 (0.70)	−0.22 (−0.37 to −0.06)	0.007	0.31
	Male ALS	3.32 (0.70)			
	Female control	3.17 (0.71)	−0.34 (−0.49 to −0.19)	<0.001	0.48
	Female ALS	3.51 (0.71)			
4. Neuroticism	Male control	2.60 (0.67)	0.08 (−0.08 to 0.25)	0.32	
	Male ALS	2.52 (0.75)			
	Female control	2.77 (0.64)	0.20 (0.06 to 0.34)	0.005	0.30
	Female ALS	2.57 (0.69)			
5. Openness	Male control	3.52 (0.54)	−0.05 (−0.18 to 0.07)	0.39	
	Male ALS	3.57 (0.56)			
	Female control	3.47 (0.53)	−0.01 (−0.13 to 0.10)	0.82	
	Female ALS	3.48 (0.62)			

Note. ALS: amyotrophic lateral sclerosis; *SD*: standard deviation; CI: confidence interval.

4 | DISCUSSION

We sought first to find out whether personality differences existed between our men and women respondents. Women scored higher than men for Agreeableness and Neuroticism, as has been reported in other studies (Weisberg, Deyoung, & Hirsh, 2011), so to compare ALS and control individuals, we analyzed male and female respondents separately. The mean Big Five scores for ALS males were higher for Extraversion and Conscientiousness. For ALS females, the scores were higher for Agreeableness, Conscientiousness, and Extraversion, and lower for Neuroticism. These particular ALS traits taken together would accord with many people's concept of what constitutes "niceness." For example, the Oxford English Dictionary provides the following synonyms for "nice" when referring to a person: "pleasant, likeable, agreeable, personable, charming, delightful, amiable, affable, friendly, kindly, genial, congenial, good-natured, engaging, gracious, sympathetic, understanding, compassionate, good" (https://en.oxforddictionaries.com/definition/nice). Several descriptors in this list could be applied to our findings in ALS of increased Agreeableness, Extraversion, and Conscientiousness, as well as decreased Neuroticism. These personality traits could therefore underlie the widespread perception, in particular among treating physicians, that people with ALS are unusually pleasant, even in the face of a devastating disease (Mehl et al., 2017).

None of the Big Five scores for ALS respondents correlated significantly with the ALS Functional Rating Scale scores, indicating that disease severity at the time of taking the questionnaire did not influence their recall of items used to measure the personality factors. Similarly, no correlation was found between any of the Big Five scores and disease duration, indicating that time elapsed after disease onset did not affect the reporting of premorbid personality scores.

The fact that ALS individuals in this study were more likely than people without ALS to have higher Agreeableness, Conscientiousness, and Extraversion, and lower Neuroticism, may have implications for the pathogenesis of the disease. Personality has a major genetic component (Vukasovic & Bratko, 2015), and it has been suggested that the same genetic factors that give rise to personality could predispose people to developing ALS (Grossman et al., 2006). Increasing numbers of studies have linked individual personality traits (in particular extraversion, which was strongly associated with ALS in the present study) with genomic loci (Lo et al., 2017). It may therefore be worthwhile examining the frequency of polymorphisms in these loci in the large genomic databases of people with and without ALS that are now available.

One possible link between personality traits and ALS is that the premorbid personality differences seen in people with ALS could lead to behaviors that are reported to be risk factors for the disease (Figure 7). Connections between personality traits and ALS risk

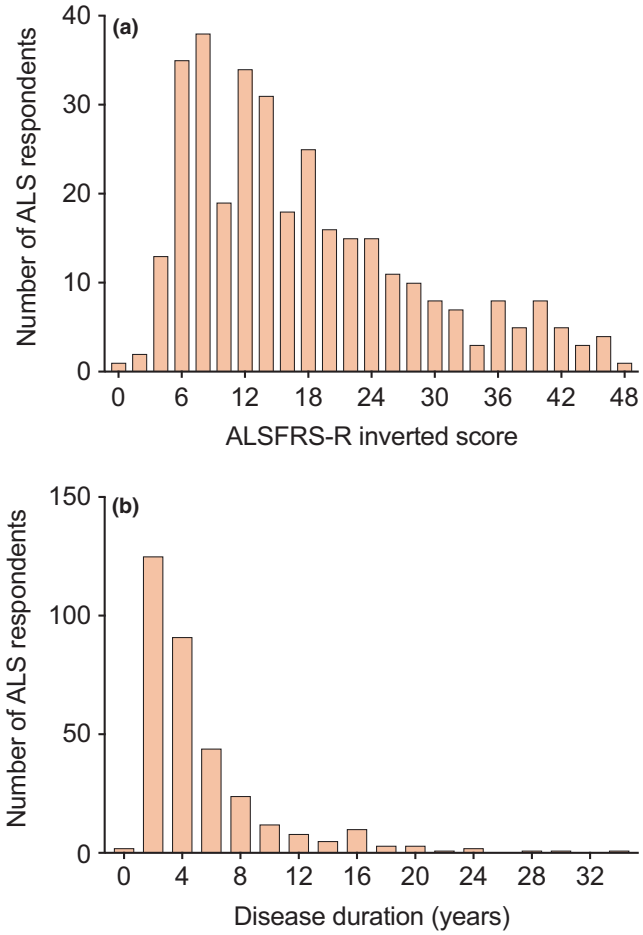

FIGURE 4 ALS functional state and duration. (a) Most ALS respondents were in the mild or moderate functional impairment range (on the left side of the histogram) as measured by the ALS Functional Rating Scale (ALSFRS-R) inverted score. A few respondents (on the right side of the histogram) were severely affected. (b) The majority of ALS respondents replied to the questionnaire within the first four years after disease onset (on the left side of the histogram), though a few long-term survivors also completed the questionnaire (on the right side of the histogram)

factors could, for example, exist between extraversion-linked smoking (Buczkowski et al., 2017), a reported risk factor for ALS (Armon, 2009); extraversion-linked risk-taking behavior (Levenson, 1990), which could lead to the increased numbers of head injuries reported in ALS (Schmidt, Kwee, Allen, & Oddone, 2010); conscientiousness-linked physical exercise (Malinauskas, Dumciene, Mamkus, & Venckunas, 2014), another suggested risk factor for ALS (Beghi et al., 2010); conscientiousness-linked low alcohol intake (Lunn, Nowson, Worsley, & Torres, 2014), which is associated with ALS (de Jong et al., 2012); conscientiousness-linked adherence to dietary advice on increased fish intake (Lunn et al., 2014), which may be a risk factor for ALS due to mercury ingestion (Andrew et al., 2018); and either conscientiousness, openness, neuroticism, or agreeableness all of which can influence choice of occupation (Zhao & Seibert, 2006), since certain occupations have been associated with ALS (Sutedja et al., 2009). These potential links imply that some of the lifestyle habits and choices thought to be risk factors for ALS may not be risk factors per se, but rather the consequence of an underlying, substantially genetically determined, personality type.

Results of ALS risk factor studies often vary between different countries. For example, smoking is reported to be an ALS risk factor in some countries (Armon, 2009), but not in others (Pamphlett & Ward, 2012). The same is true for exercise, with variable results between countries (Lacorte et al., 2016). This puzzling feature may be related to personality, since large variations in personality, particularly in conscientiousness and neuroticism, have been described across ten world regions (Schmitt et al., 2007). This means that people in different parts of the world may be predisposed to expose themselves to dissimilar risk factors. For example, populations having higher levels of conscientiousness might be less likely to smoke and those with lower conscientiousness more likely to smoke. We do not yet have sufficient numbers of respondents from different countries to investigate the effects of nation-specific personality types on ALS, but we plan to do so when we obtain more questionnaire respondents in this ongoing project.

The limitations of online questionnaires in the investigation of ALS risk factors have recently been summarized (Parkin Kullmann & Pamphlett, 2017). Limitations specific to the present study are as follows: (a) ALS respondents were asked to remember their personality aspects that applied to them *before* they were diagnosed. This is, however, of less concern in a disease like ALS which usually has a short course (as shown by the median disease duration of 3 years in the present study), than in disorders with long courses such as multiple sclerosis, where people would need to recall these aspects many years after diagnosis. (b) A potential limitation is that people with a particular personality type, especially those with high conscientiousness, are more likely to complete a detailed questionnaire. This would, however, apply to both ALS respondents and controls and should not therefore affect the comparative results. (c) Our ALS respondents provided a questionnaire response at only one time point in their disease course, so we are not able to determine whether premorbid personality had an effect on the rate of disease progress or the total duration of disease until death. However, others have studied the effects of post-ALS onset psychological factors and disease course (Krampe et al., 2008; van Groenestijn, Kruitwagen-van Reenen, Visser-Meily, Berg, & Schroder, 2016). (d) People with ALS-FTD are often prone to disinhibited behavior, which might be regarded as extraversion. However, only 2% of our ALS respondents reported being diagnosed with ALS-FTD, so the inclusion of this group is unlikely to have had a significant effect on the overall results. Furthermore, it is unlikely that respondents with moderate or severe dementia would be capable of completing the detailed questionnaire. (e) Numbers of respondents in the nonclassical ALS subgroups (progressive muscular atrophy, primary lateral sclerosis, progressive bulbar palsy, and ALS/FTD) were too small, when separated by gender, to assess whether personality differences were statistically different in these individual subgroups. (f) We have no way of telling if some people with ALS, most likely due to

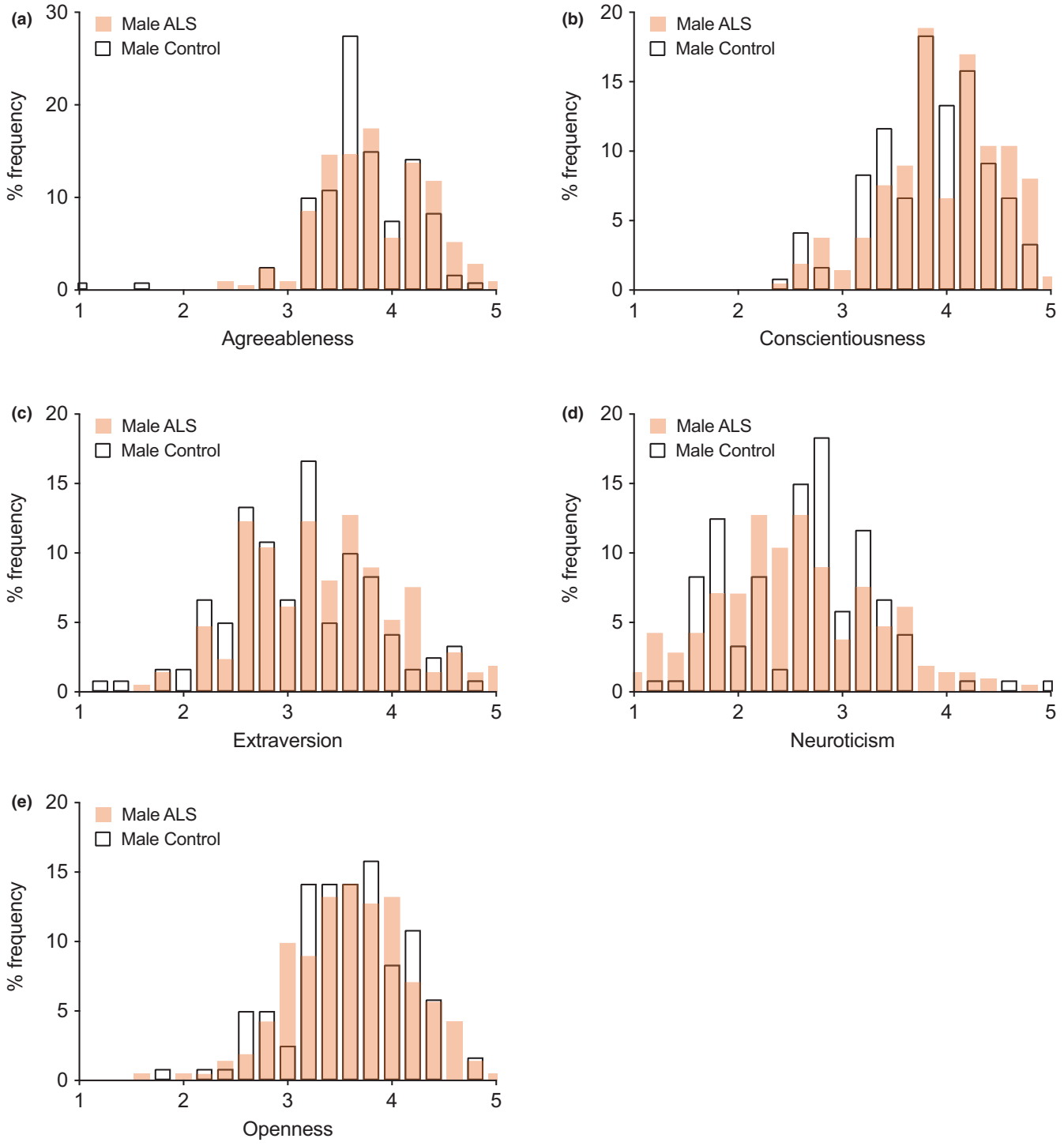

FIGURE 5 Percentage frequency distributions of male ALS and control individuals. The two distributions overlap, but male ALS distributions are shifted to the right for Agreeableness, Conscientiousness, and Extraversion, and slightly to the left for Neuroticism

muscle weakness, needed help from another person to complete the questionnaire. The questionnaire format was compatible with computer assistive technologies such as voice-activated systems, but some people with bulbar weakness would not have been able to use this. We think, however, that having help to complete the questionnaire would not affect the results. (g) There is a possibility that non-ALS controls over the age of 40 years could develop ALS later. However, ALS is a relatively uncommon disorder, with a lifetime risk of about 1:350 for men and 1:400 for women (Van Es et al., 2017), so since we had 120 male and 282 female controls, only one of our controls in total is likely to develop ALS during their lifetime, which would not affect the results.

In conclusion, people with ALS appear more likely than controls to have personality traits that can be considered to represent "niceness."

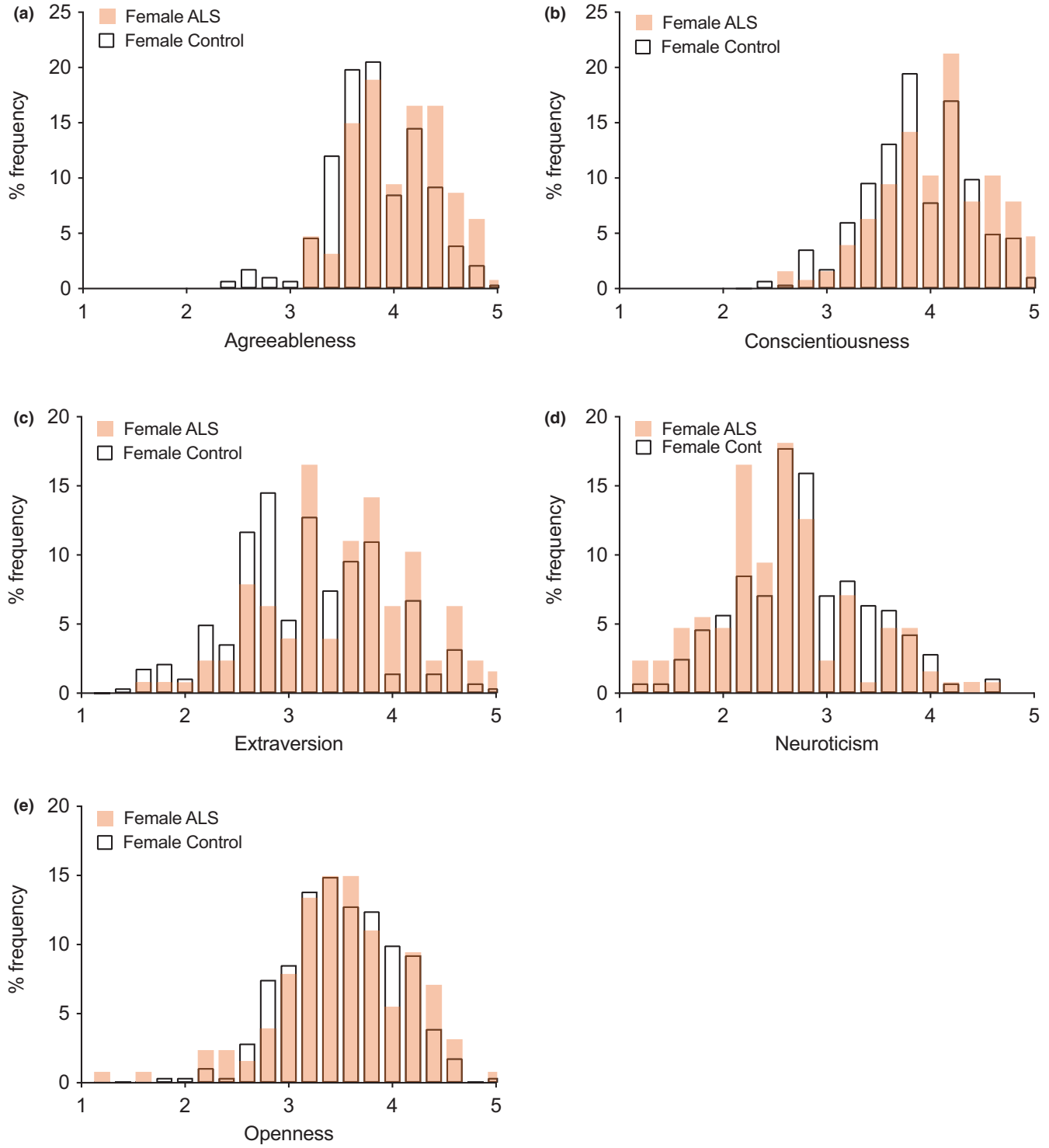

FIGURE 6 Percentage frequency distributions of female ALS and control individuals. The two distributions overlap, but female ALS distributions are shifted to the right for Agreeableness, Conscientiousness, and Extraversion, and to the left for Neuroticism

Possibilities that need to be considered are whether personality differences in ALS influence lifestyle choices (and therefore disease risk factors) and whether genetic variants influencing personality could be associated with ALS itself. To take into account the influence of personality on lifestyle choices, we recommend that personality testing be included in future investigations of risk factors for ALS.

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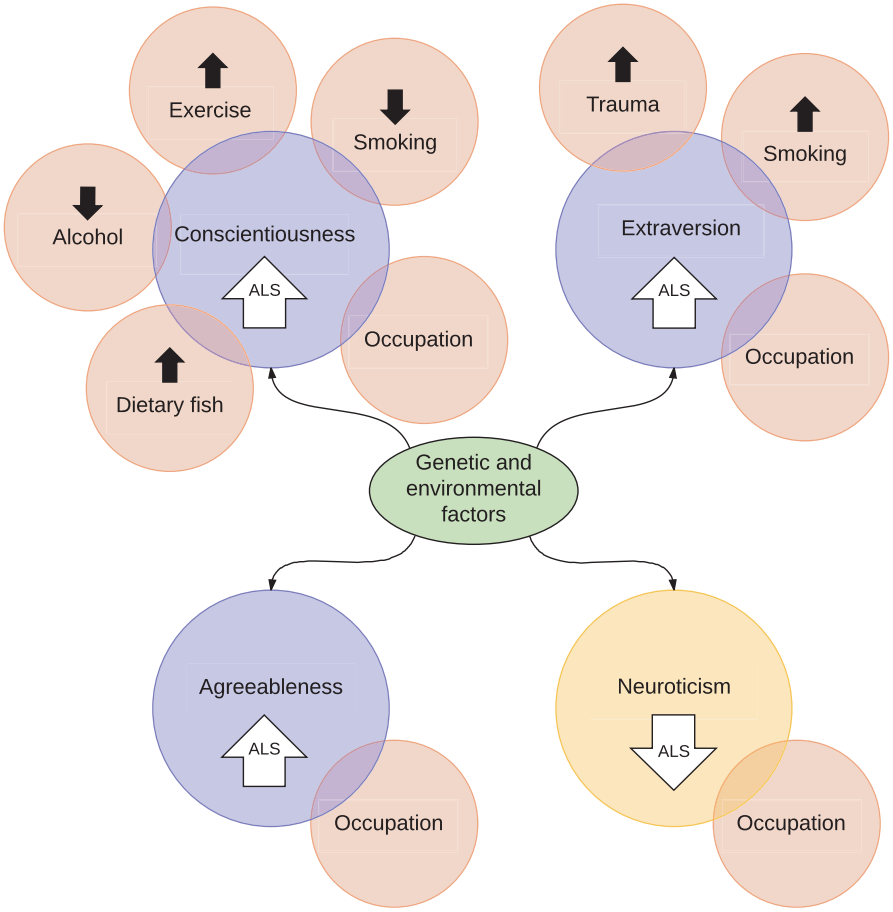

FIGURE 7 Potential interactions between the four ALS-related personality traits described in the present study (large circles) and postulated ALS risk factors (small circles). Both genetic and environmental factors underlie personality traits. Increased Conscientiousness in ALS could be associated with increased exercise, decreased alcohol intake, and increased fish consumption, all possible ALS risk factors. Increased Extraversion could be associated with increased smoking and increased risk-taking behavior leading to head trauma, both reported ALS risk factors. All four personality factors could influence choices of occupations, with some occupations being associated with the risk of ALS

the USA CDC National ALS Registry, and the Canadian Neuromuscular Disease Registry assisted in recruiting respondents for the questionnaire. The questions used in the ALS Quest questionnaire for this project can be viewed online at www.alsquest.org. Data used in the analyses will be made available to any researcher, for purposes of reproducing the results or replicating the findings, via email attachments of Microsoft Excel spreadsheets. The research analysis plan was not preregistered. The authors declare no conflict of interests. This study is supported by the Aimee Stacey Memorial and the Ignacy Burnett Bequests.

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Chapter 5: Is psychological stress a predisposing factor for amyotrophic lateral sclerosis (ALS)? An online international case-control study of premorbid life events, occupational stress, resilience and anxiety

Another novel risk factor that was investigated as part of this project was how psychological stress might be related to ALS. Interest in this factor arose due to i) a potential increased uptake of toxins due to exposure to stress [1], ii) a possible link between the biological effects of stress and the mechanism by which ALS develops [2], and iii) the anecdotal reporting from ALS patients of stress as a contributing cause of their disease.

A link between stress and ALS has never been systematically explored. Only one study has looked at stress in ALS, and that was in a cursory fashion [3]. Therefore, there was a need for a robust analysis of the relationship of stressful life events with ALS.

To evaluate stress and its role in ALS, the questionnaire included an inventory of stressful life events – a modified version of the Social Readjustment Rating Scale [4] – which indicated the lifetime stress experienced by respondents. The study also assessed whether resilience or anxiety affected the levels of stress experienced by respondents.

The results in the publication in this chapter indicate that the levels of stress experienced by ALS respondents were not higher those experienced by controls. In fact, some controls had higher exposure to stress than people with ALS. Furthermore, ALS patients had higher resilience, indicating the potential for a better response to stress when it did occur. Therefore, this study does not support stress as a risk factor for ALS.

These results were presented in a platform presentation at the 29th International Symposium on ALS/MND in 2018 (Appendix I).

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PLOS ONE

RESEARCH ARTICLE

Is psychological stress a predisposing factor for amyotrophic lateral sclerosis (ALS)? An online international case-control study of premorbid life events, occupational stress, resilience and anxiety

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Abstract

Psychological stress has been suggested to be relevant to the pathogenesis of neurodegenerative disorders, possibly via the generation of oxygen free radicals. We therefore sought to determine whether people with amyotrophic lateral sclerosis (ALS) had been subjected to more potentially stressful life events or occupations than controls, and whether they had differences in resilience or trait anxiety that would moderate their responses to these stressors. An online anonymous multilingual questionnaire was used to collect data on significant life events from people with and without ALS, using items from a modified Social Readjustment Rating Scale and from self-described significant events, which were combined to create a Life Events Inventory. Inventory scores were subdivided into 0–20 years and 21–40 years age ranges, and for the preceding 2, 5 and 10 years. Respondents also rated levels of stress experienced during different occupations. Resilience was measured using the Connor-Davidson Resilience Scale, and trait anxiety with a modified Geriatric Anxiety Inventory. Scores were compared using nonparametric statistics. Data from 400 ALS (251 male, 149 female) and 450 control (130 male, 320 female) respondents aged 40 years and over showed that Life Events Inventory scores were similar in male ALS respondents and controls, but lower in female ALS respondents than female controls for the preceding 5-year and 10-year periods. Occupational stress did not differ between ALS respondents and controls. Both male and female ALS respondents had higher resilience scores than controls. Anxiety scores did not differ between ALS and control groups. In conclusion, people with ALS reported no raised levels of potentially stressful premorbid life events or occupational stress, and did not have reduced levels of resilience, or increased levels of anxiety, that would augment the deleterious effects of stressors. On the contrary, ALS respondents had higher resilience than controls, though this conclusion relies on ALS respondents recalling their premorbid status. These results do not support the hypothesis that psychological stress from significant life events or occupational stress plays a role in the pathogenesis of ALS.

Introduction

Stress can be defined as the response of an organism to stressors such as changes in a work situation, a death in the family, or moving house [1,2]. Stress experienced as the result of an external stressor can be irrespective of its perceived negativity or positivity; for example, marriage and divorce are two of the most impactful stressors [3]. The primary filter for understanding and responding to stressors is the brain, which first processes the stressor and then creates a response (ie, the stress), which can include adaptive physiological or psychological changes to the individual [4]. Physiological components of the response include increased oxidative stress, which can lead to tissue damage in a chronically-stressed individual [5,6].

Stress has been proposed as a risk factor for a number of neurological diseases. For example, stressful life events appear to be more frequent in people with multiple sclerosis [7,8] and stressful life events that compromise the immune system may be associated with the onset of multiple sclerosis [9]. Midlife psychological stress may be a risk factor for the development of Alzheimer disease and other form of dementia [10]. Chronic restraint stress in rodents triggers dopaminergic and noradrenergic degeneration, a pattern of cell loss that is characteristic of Parkinson disease [11]. Overall, however, there is not a strong body of human evidence to support the concept of psychological stress being a trigger factor for these common neurological diseases.

In amyotrophic lateral sclerosis (ALS, also known as motor neuron disease) stressors could increase the uptake of neurotoxins, such as mercury, into a stress-activated locus ceruleus, with a subsequent decrease in noradrenaline output to the brain and spinal cord [12]. Another possible connection between stress and ALS is reduced telomerase activity and telomere shortening due to stress in early life [13]. Only one study has looked for links between premorbid psychological stress and ALS. In this study, either low or high self-reported stress was considered in combination with personality type (A or B) and factors that might increase oxidative stress (eg, physical activity and smoking), as well as those that might reduce oxidative stress (eg, consumption of vegetables) [14]. Findings were that high stress, a type A personality, and physical activity were present more often in people with ALS. However, this study did not use any standard measurements of stress or personality, and results were not compared between genders [15].

In an attempt to determine whether people with ALS have been subjected to more significant life events (and therefore more potential stressors) or workplace stressors than controls, and whether they have different levels of resilience or anxiety that could affect their responses to these stressors [16], we asked ALS and non-ALS participants to complete a web-based questionnaire with items designed to measure the occurrence of significant life events, occupational stress, and levels of resilience and anxiety.

Methods

Setting

This case-control study used data collected between January 2015 and September 2017 from a multilingual web-based questionnaire, ALS Quest [17]. The questionnaire, which uses Qualtrics survey software, can be viewed at www.alsquest.org. Respondents for the questionnaire were recruited via worldwide national and state ALS Associations, national ALS registries, the Internet and social media. No personally-identifying data were collected so respondents remained anonymous. Information on disease status was self-reported. Cases were respondents who stated 'Yes, I have been diagnosed with ALS/MND.' Controls were participants who stated 'No, I have not been diagnosed with ALS/MND.'

Online consent

Participants consented to submit their questionnaire responses for inclusion in the study by clicking an 'I consent' button, after agreeing that 'I: 1. Acknowledge that I have read the Information for Participants above and agree to participate in the research, 2. Understand that I will not be asked for any personal information that could identify me, so the study is anonymous and strictly confidential, and 3. Freely choose to participate in the study and understand that I can withdraw my questionnaire answers at any time until I click the Submit button at the end of the questionnaire.'

Ethics

The project, 'ALS-Quest: An online questionnaire for research into amyotrophic lateral sclerosis' (X14-357), was approved by the Human Ethics Committee of the Sydney Local Health District (RPAH Zone).

Overview of model to estimate lifetime psychological stress

To estimate lifetime psychological stress, we devised a method to first identify significant life events that could give rise to stress, as well as occupational stress, and then to measure personality factors that could either reduce or increase the amount of stress induced (Fig 1).

Life events

Pre-defined life events: Modified Social Readjustment Rating Scale (mSRRS). The 1967 SRRS list consists of 43 events, ranging in severity from 'death of a spouse' (rated as 100) to 'minor violations of the law' (rated as 11) [3], and is the most widely used instrument for the

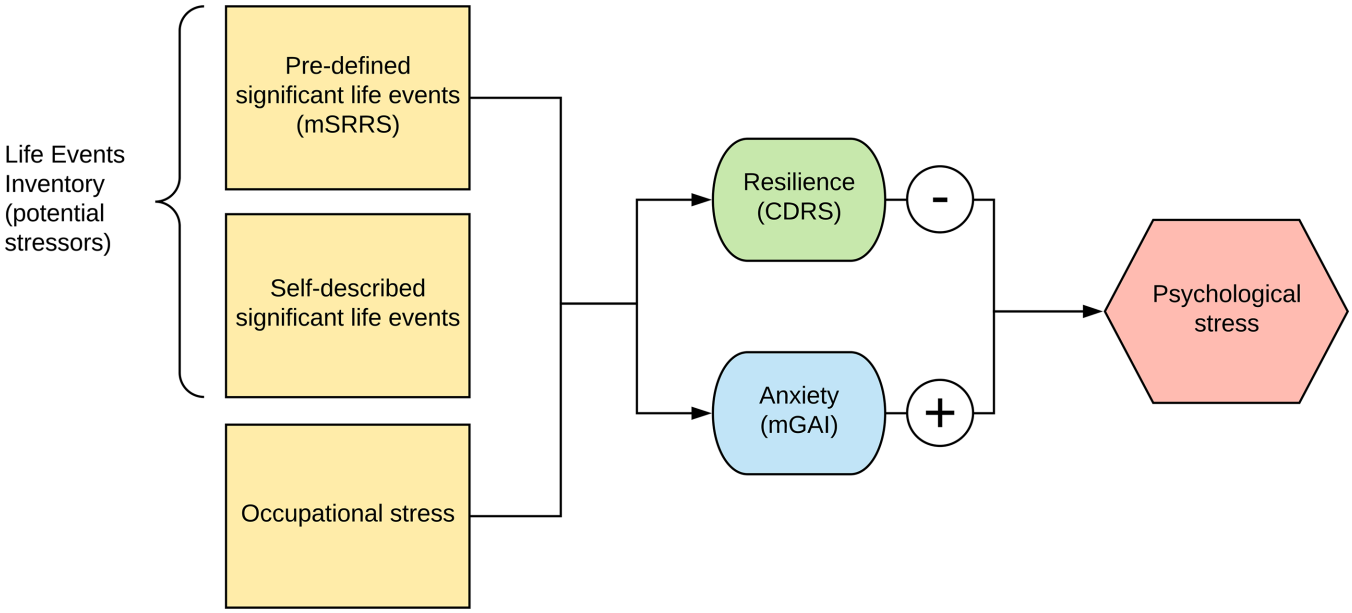

Fig 1. Method to estimate lifetime psychological stress. Lifetime stressors can arise from potentially-stressful significant life events, identified either from a pre-determined list or by those described by respondents, and self-reported occupational stress. A high level of resilience would tend to reduce the amount of psychological stress induced by these stressors, whereas high levels of anxiety would tend to increase psychological stress. CDRS: Connor-Davidson Resilience Scale, mGAI: modified Geriatric Anxiety Inventory, mSRRS: modified Social Readjustment Rating Scale.

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measurement of a person's experience of psychological stress [18]. To develop the ratings in the SRRS, respondents are asked to score events based on the amount of 'social readjustment' required per event, judging by their experience and the experiences of people they knew, irrespective of the age at which the event occurred and the number of times it occurred. Use of the SRRS for measuring stress has been criticised on some grounds, including the fact that a particular event, eg, marriage, could be either stressful or enjoyable. However, stress experienced as the result of an external stressor can be irrespective of its perceived negativity or positivity [3]. Nevertheless, a number of modifying variables have been sought that could moderate the capacity of a significant life event to cause stress, such as asking respondents to rate the desirability of each item [18,19]. We did not ask our respondents to do this, because this would introduce an unacceptable level of subjectivity to the responses since respondents would have to try to remember their feelings about events throughout their lives, and not only in the last 12 months as is usual for studies using the SRRS.

Having pre-defined events as a measure of stress enables our method to be compared with those in other neurological disorders such as multiple sclerosis where stress has been implicated as a disease trigger [9]. Therefore, to provide an objective measure of stress, we did not exclude events (except a few noted below) and we did not adjust or weight our results [18]. Instead, we modified the SRRS by: (1) omitting items used to assess stress in the preceding year only (ie, 'Revision of Personal Habits,' 'Vacation,' and 'Christmas'), (2) allowing respondents to report up to five occurrences of each significant event, and to select their age when each event occurred, (3) Separating the results for men and women, since there are gender differences in the incidence of ALS [20], occupational choices, types of significant life events [21] and stress and coping styles [15], and (4) Updating monetary amounts quoted in the mSRRS to 2014 values, based on the inflation rate in the United States of America since 1967 (http://www.usinflationcalculator.com/).

Self-described events. Respondents were invited to list up to five significant events, not covered in the mSRRS, that had occurred in their lives, and the age at each occurrence. These events were scored as 36 points, the average score of the mSRRS events.

The scores from the mSRRS items and the Self-Described Events were added to give a Life Events Inventory (ie, potential life event stressors) score. ALS respondents were asked to include only events occuring *before* their age of diagnosis. For controls, all events were included. For example, a 62 year-old ALS respondent, diagnosed when 58 years old, who had selected the three mSRRS items 'I got married' (50 points) when 33 years old, 'I changed to a different occupation' when 42 years and 51 years old (each 36 points), and 'My sleeping habits changed significantly' (16 points) when 60 years old, and three Self-Described Events at ages 23, 46 and 50 years (36 points per event), would have a Life Events Inventory score of 230, ie, 50+36+36+(36x3). Note that this calculated total excludes the event that occurred at age 60 years because this was after the age at which ALS was diagnosed.

Life Events Inventory scores were calculated for all events, as well as for events occurring during the timespans of 0–20 years and 21–40 years, and for events that had occurred during the previous 2, 5 and 10 years before ALS diagnosis. The 2-year time period before diagnosis was chosen to assess whether stressors occurring a short time before the onset of ALS could trigger the disease. The average period of time from clinical onset of ALS to the time a neurologist makes the diagnosis (the diagnostic delay) is close to one year [22]. An ALS respondent would therefore have to report any life events that had occurred two years before diagnosis to ensure that the one year of pre-disease-onset events were included. Control respondents were also asked to report events in the same 2-year period, to ensure consistency between groups as regards the recall of events.

Occupational stress

Respondents were asked to document all occupations they had held for six months or longer, and to report the level of stress associated with each. Answers were rated as 0 for none, 1 for mild, 2 for moderate and 3 for severe stress. Scores were multiplied by the number of years worked in each occupation, summed, and divided by the total number of years in the occupations to estimate overall occupational stress. For example, a person who had worked at a mildly stressful occupation for 30 years and a moderately stressful occupation for 10 years would have a score of 1.25, ie, [(30x1) + (10x2)]/40.

Resilience

The Connor-Davidson Resilience Scale (CDRS) consists of 25 statements which respondents rated on a 5-point scale from 'strongly disagree' to 'strongly agree' [23]. Answers were scored from 0 to 4 to create a total score that ranged from 0 to 100, with higher numbers denoting greater resilience. People with ALS were asked to provide responses they would have given to the statements *before* their ALS diagnosis.

Anxiety. The original Geriatric Anxiety Inventory consists of 20 statements asking how respondents felt (eg, 'I worry a lot of the time') in the month before completing the Inventory, and scored as Yes (1) or No (0) to give a score ranging than 0 to 20, with lower numbers indicating a lower level of anxiety [24]. The Geriatric Anxiety Inventory was modified for this project by asking respondents to rate each statement on a 5-point scale from 'strongly disagree' (scored as 0) to 'strongly agree' (scored as 4), giving a final score that ranged from 0 to 80. ALS respondents provided responses they would have given to the modified Geriatric Anxiety Inventory (mGAI) statements in a typical month *before* their ALS diagnosis.

Exclusion criteria

Respondents were excluded from analysis (Fig 2) if they did not complete at least one of the inventories (Life Events Inventory, CDRS, or mGAI), they did not supply their age, or they were under the age of 40 years at the time of completing the questionnaire; the latter was to limit the number of control respondents who might later develop ALS, and to limit different life event perceptions and frequencies between younger and older individuals [25].

Data analysis

Data from the Qualtrics server were transferred to GraphPad Prism 7 files. Some variables were not normally distributed so non-parametric Mann-Whitney U tests were used to compare two groups. Non-parametric effect sizes (r) were calculated as z/sqrt(N). Significance was assessed at the 0.05 level.

Results

Cases and controls

850 respondents remained after exclusion criteria were applied (Fig 2). These comprised 400 ALS respondents (251 male, 149 female) and 450 non-ALS controls (130 male, 320 female). The mean age of ALS respondents (male and female combined) was 61.5 years (SD 9.2 years, range 40–87 years) and of controls was 57.3 years (SD 10.5 years, range 40–89 years), a significant difference. Male ALS respondents (mean age 62.0 years) and male controls (mean age 61.8 years) did not differ significantly in age. Female ALS respondents (mean age 60.7 years) were significantly older than female controls (mean age 55.5 years).

Common sources of information about the questionnaire cited by respondents were: ALS Associations (39%), the Internet (21%), friends (9%), ALS patients (6%), the USA CDC National ALS Registry (5%), health professionals (5%), community groups (4%), Facebook (4%), the Canadian Neuromuscular Disease Registry (2%), and ALS researchers (2%).

The composition of ALS and control groups was similar with regards to country of birth, country of residence, ancestry and cultural group (Table 1). The majority of respondents resided in Australia, the USA and Canada, though residents of a further 29 countries supplied responses.

Clinical characteristics

Sporadic and familial ALS. Nine percent of ALS respondents had at least one relative who had been diagnosed with ALS and were deemed to have familial ALS. The other 91% were categorised as having sporadic ('isolated') ALS.

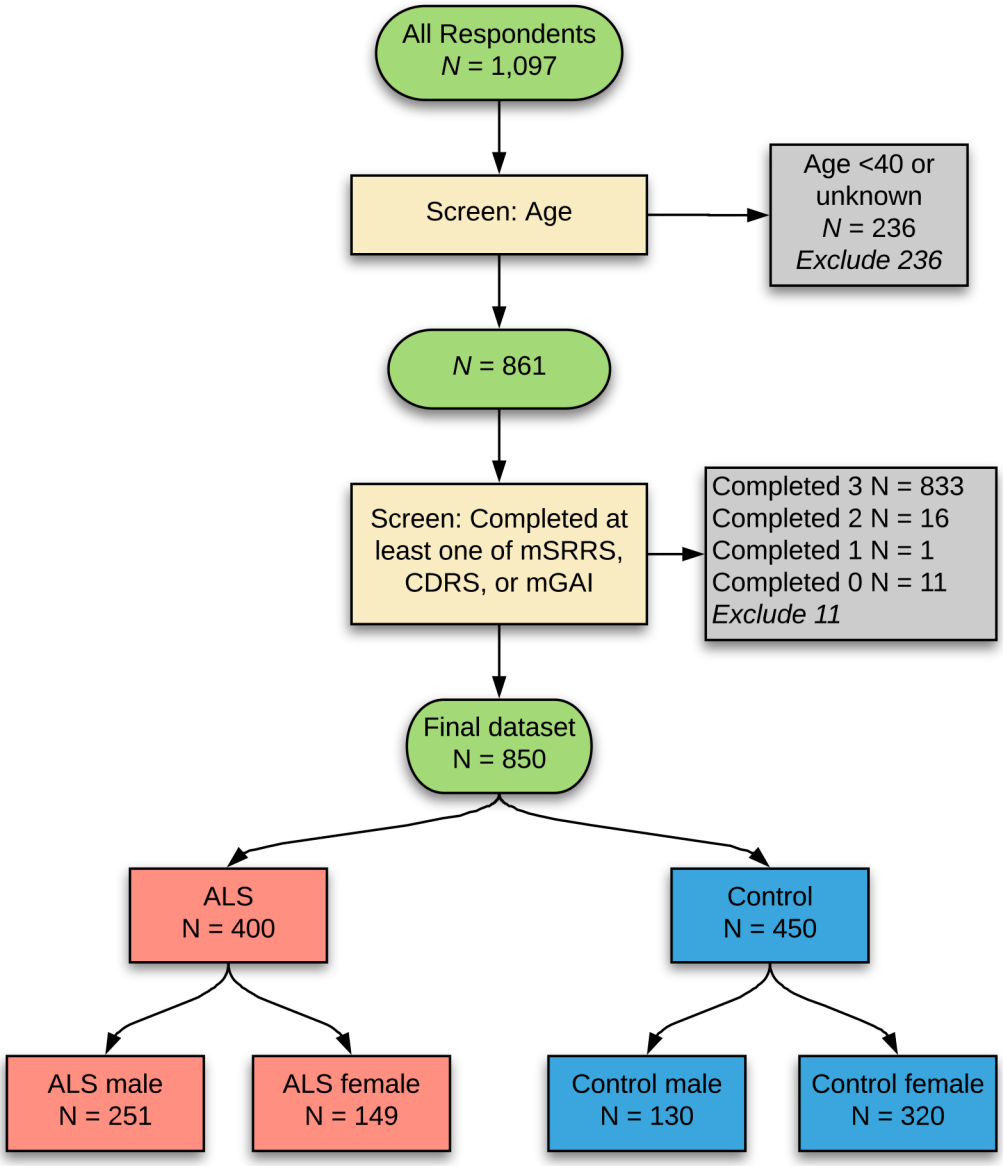

Fig 2. Selection of individuals for analysis. The final dataset of 850 respondents was obtained after exclusion criteria were applied for younger age and not completing at least one of the stress-related items.

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Table 1. Demographic characteristics of respondents.

	ALS	Number (%)	Control	Number (%)
Country of birth				
	United States	170 (43%)	Australia	276 (62%)
	Australia	94 (24%)	Other (<2% each)	73 (16%)
	Canada	49 (12%)	United States	42 (9%)
	Other (<1% each)	57 (14%)	United Kingdom	28 (6%)
	United Kingdom	18 (5%)	New Zealand	15 (3%)
	Spain	10 (3%)	Spain	14 (3%)
Country of residence				
	United States	179 (45%)	Australia	338 (75%)
	Australia	116 (29%)	United States	48 (11%)
	Canada	57 (14%)	Other* (<2% each)	36 (8%)
	*Other (<2% each)	37 (9%)	Spain	14 (3%)
	Spain	9 (2%)	New Zealand	12 (3%)
Ancestry				
	Other (<6% each)	184 (47%)	Other (<4% each)	153 (34%)
	Australian	60 (15%)	Australian	130 (29%)
	English	57 (15%)	English	77 (17%)
	American	36 (9%)	Irish	40 (9%)
	German	32 (8%)	British	25 (6%)
	Irish	23 (6%)	Scottish	19 (4%)
Cultural group				
	American	111 (29%)	Australian	285 (64%)
	Australian	98 (26%)	Other (<2% each)	76 (17%)
	Other (<3% each)	93 (24%)	American	32 (7%)
	Canadian	40 (10%)	English	27 (6%)
	English	28 (7%)	Spanish	14 (3%)
	German	13 (3%)	New Zealander	11 (2%)

Other* (countries of residence): Argentina, Belgium, Brazil, Cape Verde, China, Colombia, Czech Republic, Denmark, Ecuador, Egypt, Finland, Germany, Iran, Ireland, Italy, Luxembourg, Mexico, Netherlands, Portugal, Russia, Slovakia, South Africa, South Korea, Sweden, Switzerland, Turkey, United Kingdom.

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Subtypes of ALS. 58% of ALS respondents had 'classic' (upper and lower motor neuron variant) ALS, 9% progressive muscular atrophy (lower motor neuron variant), 9% progressive bulbar palsy, 8% primary lateral sclerosis (upper motor variant), 2% ALS/frontotemporal dementia, 6% 'other', and 8% did not know their subtype of ALS.

ALS functional rating scale. People with ALS were asked to complete the ALS Functional Rating Scale-Revised designed for online use [26] to assess their physical state at the time of taking the questionnaire. Scores were inverted from the standard scale so that the higher the score the more impaired the function, with 0 indicating no impairment and 48 indicating very severe impairment. The median Functional Rating scale was 13, and this and the distribution of the rating scores (Fig 3A) are those expected for a generally rapidly progressive disease like ALS [22].

Duration of ALS. The duration of ALS at the time of completing the questionnaire was calculated by subtracting the year of diagnosis from the year of consenting to complete the questionnaire. The median duration of disease was 1 year, and this, and the disease duration distribution (Fig 3B), are those expected in a usually short-lasting disease like ALS [22].

Controls. Control participants comprised (blood or non-blood) relatives (45%), friends of ALS respondents (13%), partners (including spouses) (10%), and individuals from

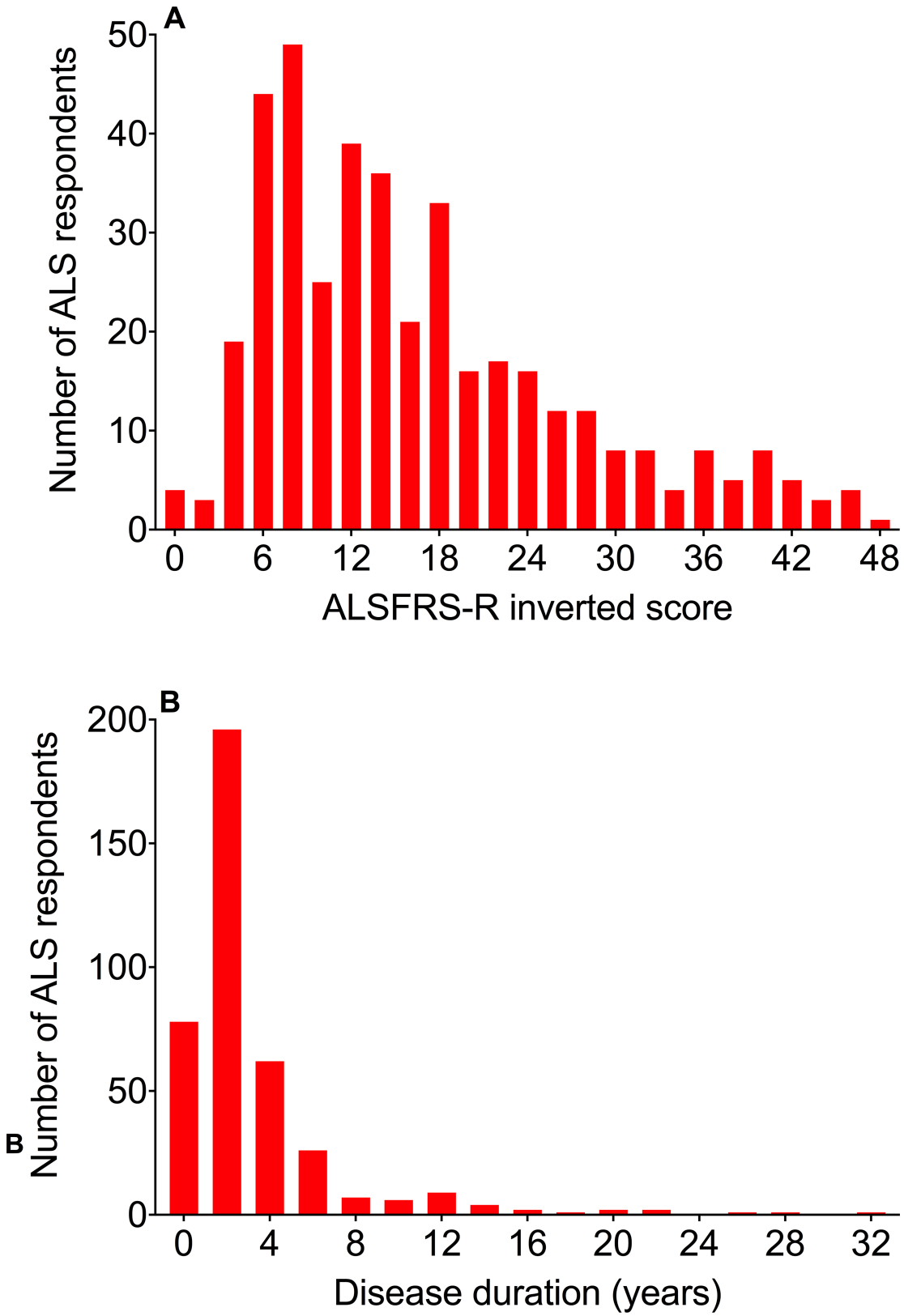

Fig 3. Disability and disease duration in ALS respondents. (A) Most ALS respondents had mild or moderate disability (with lower ALSFRS-R inverted scores, on the left), while fewer had severe disability (higher ALSFRS-R inverted scores, on the right).

(B) Most ALS respondents completed the questionnaire within four years of being diagnosed. A small number of long-term ALS survivors (on the right) also completed the questionnaire.

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community, research, and medical professional groups (9%). 23% of controls reported either no specific or another type of connection to ALS. Most controls (56%) reported no blood relatives with ALS, whereas 32% had one, and 12% more than one, blood relative/s with ALS.

Life events inventory

Males and females. Females had higher Life Events Inventory scores than males for all ages combined, for the age ranges of 0–20 years and 21–40 years, and for the previous 2, 5 and 10 years (Table 2). Effect sizes ranged from 0.12 (for the previous 5 years) to 0.18 (for the age range 21–40 years).

ALS and controls. No differences in Life Events Inventory scores were found between male ALS and male control respondents for all ages combined (Figs 4A and 5A), for the age ranges of 0–20 years and 21–40 years, or for the previous 2, 5 and 10 years (Table 3). Life

Table 2. Male-female comparisons in scores for the life events inventory, occupational stress, resilience and anxiety.

Respondents (number)	Median	Mean rank	Mann-Whitney *p*	Effect size *r*
Life Events Inventory				
All ages				
Male (370)	659	380	<0.001*	0.15
Female (467)	748	450		
Age 0–20 y				
Male (370)	66	383	<0.001*	0.13
Female (467)	106	447		
Age 21–40 y				
Male (370)	281	371	<0.001*	0.18
Female (467)	345	457		
Previous 2 y				
Male (370)	20	386	<0.001*	0.13
Female (467	39	445		
Previous 5 y				
Male (370)	70	387	0.001*	0.12
Female (467)	92	444		
Previous 10 y				
Male (370)	136	380	<0.001*	0.15
Female (467)	185	450		
Occupational Stress				
Male (363)	1.90	432	0.005*	0.10
Female (449)	1.81	386		
Connor-Davidson Resilience Scale				
Male (378)	74	413	0.24	0.04
Female (469)	75	433		
Modified Geriatric Anxiety Inventory				
Male (379)	20	400	0.012*	0.08
Female (469)	22	444		

*: $p<0.05$

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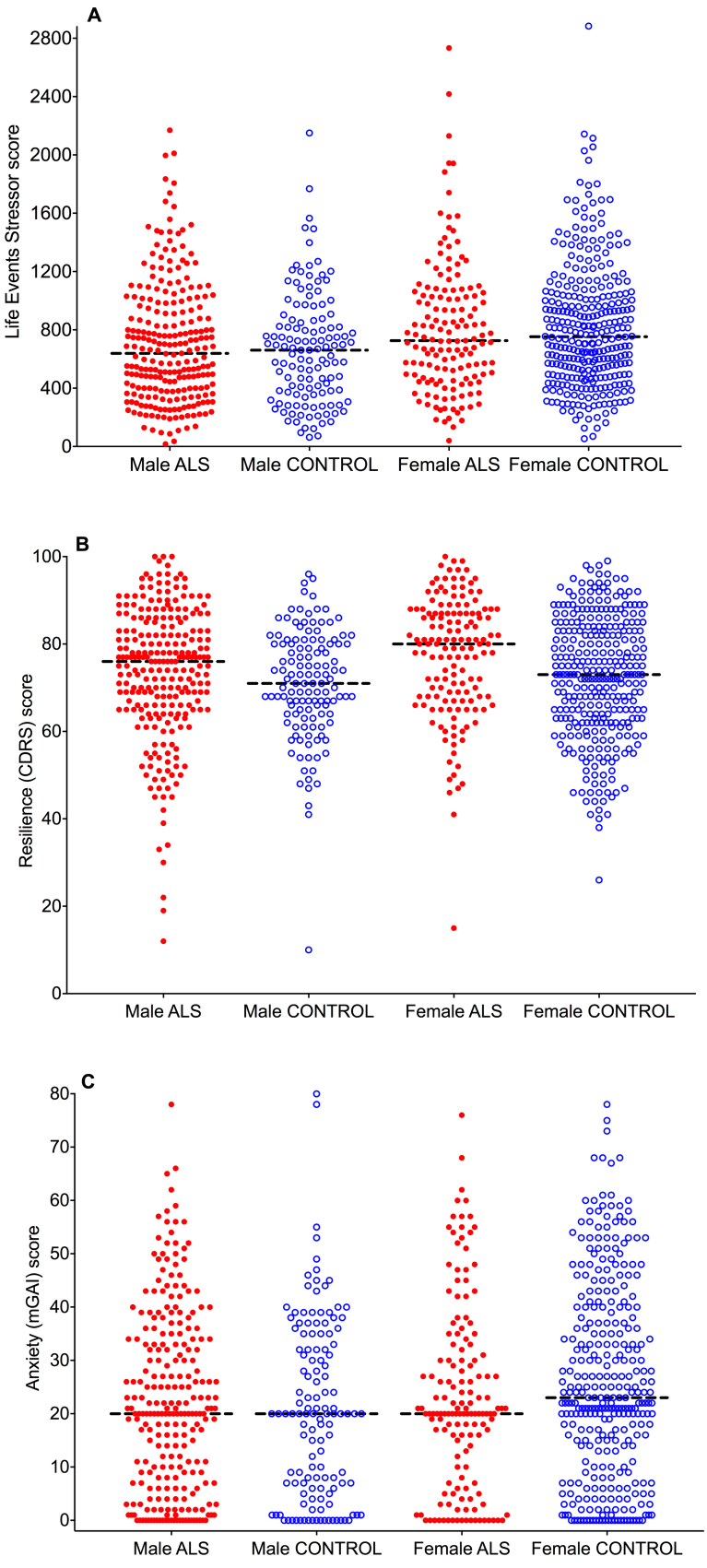
A
Life Events Stressor score
2800
2400
2000
1600
1200
800
400
0
Male ALS
Male CONTROL
Female ALS
Female CONTROL
B
Resilience (CDRS) score
100
80
60
40
20
0
Male ALS
Male CONTROL
Female ALS
Female CONTROL
C
Anxiety (mGAI) score
80
70
60
50
40
30
20
10
0
Male ALS
Male CONTROL
Female ALS
Female CONTROL

Fig 4. Distributions and median values of scores for the Life Events Inventory (all ages), resilience and anxiety. (A) The distribution and median values of Life Events Inventory scores are similar between male and female ALS and control respondents. (B) Both male and female ALS and control respondents have higher median values for resilience (CDRS scores) than their gender controls. (C) Male ALS and control respondents have similar distributions and median values for anxiety (mGAI scores), while female ALS respondents have lower anxiety levels than female controls. CDRS: Connor-Davidson Resilience Scale, mGAI: modified Geriatric Anxiety Inventory.

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Events Inventory scores were similar in female ALS respondents and controls for all ages combined (Figs 4A and 5B), but were *lower* in female ALS respondents compared to female controls for the previous 5-year and 10-year periods (Table 3).

Control groups. Life Events Inventory scores were compared between partners, relatives and others to assess the degree of homogeneity between the three different control groups. Scores between the three groups differed for the most recent 2 years ($p = 0.002$), the 5-year time point ($p = 0.002$), and the 10-year time point ($p = 0.001$). However, when each of the three control groups was compared to ALS respondents for these variables, the control group had either a higher or no significantly different median rank to the ALS group. This indicates that inter-control differences are unlikely to affect the overall ALS vs control results, and that overmatching with the use of partner controls is not a major factor [27], probably because only 10% of controls were partners.

Comparison of mSRRS and self-described events scores. Only 85 respondents (10% of the total) reported one or more self-described significant event/s. Eleven male controls (8%) had an average of 2.5, and 24 male ALS respondents (10%) an average of 1.9, Self-Described Events. Thirty-four female controls (11%) had an average of 2.8, and 16 female ALS respondents (11%) an average of 2.4, Self-Described Events. To examine the relationship between mSRRS and Self-Described Events scores, Life Events Inventory scores were calculated without Self-Described Events to remove their contribution, as well as using 100 as the score for Self-Described Events (to maximise their potential contribution, since this is the maximum possible score in the mSRRS). This did not change the outcome of the statistical tests at either of the extremes (results not shown), indicating that the outcome was not affected by inclusion of the Self-Described Events.

Occupational stress

Male respondents reported higher occupational stress than female respondents (Table 2). Occupational stress scores did not differ between male or female ALS respondents and their respective gender controls (Table 3).

Connor-davidson resilience scale

Resilience did not differ between male and female respondents, with similar CDRS scores in these gender groups (Table 2). Both male and female ALS respondents had higher CDRS resilience scores than their respective gender controls (Table 3). The differences in distributions of resilience scores and median values can be seen in Fig 4B. Frequency distribution histograms show the shift to the right of resilience scores for male and female ALS respondents (Fig 5C and 5D).

Modified geriatric anxiety inventory

Anxiety (mGAI) scores were higher in females than males (Table 2). Anxiety scores were not significantly different between male and female ALS respondents and their respective gender controls (Table 3). Distributions and median values of anxiety scores for male ALS and control

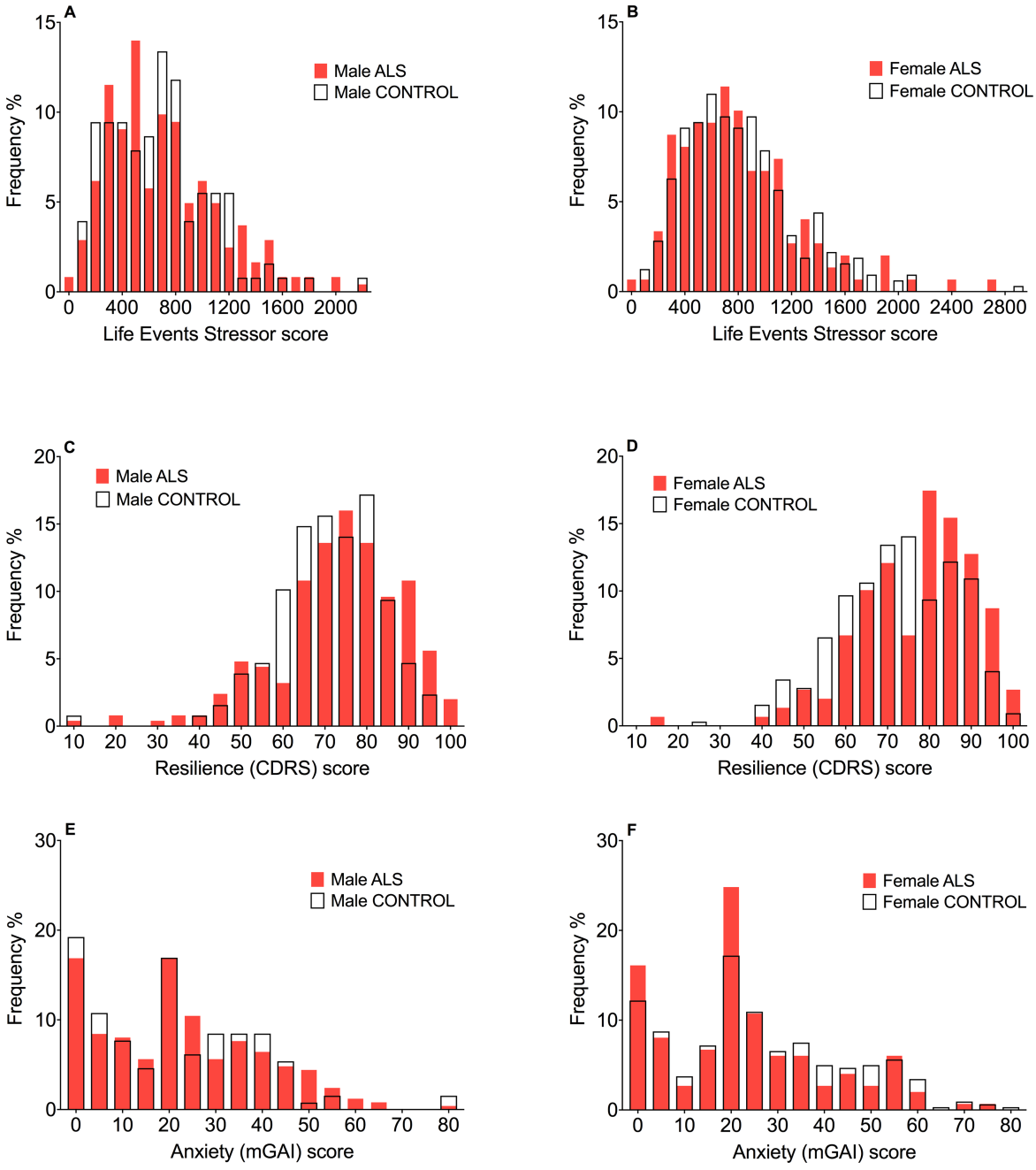

Fig 5. Frequency distributions of scores for the Life Events Inventory (all ages), resilience, and anxiety. The frequency distributions of Life Event Inventory scores are similar between ALS and control males (A), as well as between ALS and control females (B). Resilience scores (CDRS) are shifted to the right (ie, greater resilience) in both male (C) and female (D) ALS respondents. The frequency distribution of anxiety scores (mGAI) are similar in both male (E) and female (F) ALS and control respondents. CDRS: Connor-Davidson Resilience Scale, mGAI: modified Geriatric Anxiety Inventory.

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respondents (Fig 4C), and their percentage frequencies (Fig 5E), were similar. Female ALS respondents had a non-significant tendency to have *lower* anxiety levels than female controls, as seen in slight differences in anxiety score distributions and median values (Fig 4C), with the percentage frequency of ALS female anxiety scores being shifted slightly to the left (Fig 5F).

Table 3. ALS-control comparisons in scores for the life events inventory, occupational stress, resilience and anxiety.

Respondents (number)	Median	Mean rank	Mann-Whitney *p*	Effect size *r*
MALES				
Life Events Inventory				
All ages				
ALS (243)	639	188	0.528	0.03
Control (127)	661	181		
Age 0–20 y				
ALS (243)	68	189	0.364	0.05
Control (127)	65	179		
Age 21–40 y				
ALS (243)	288	189	0.342	0.05
Control (127)	268	178		
Previous 2 y				
ALS (243)	19	180	0.177	0.07
Control (127)	29	195		
Previous 5 y				
ALS (243)	63	180	0.187	0.07
Control (127)	81	196		
Previous 10 y				
ALS (243)	128	178	0.100	0.09
Control (127)	152	198		
Occupational Stress				
ALS (239)	1.92	185	0.446	0.04
Control (124)	1.89	176		
Connor-Davidson Resilience Scale				
ALS (250)	76	198	0.028*	0.11
Control (128)	72	172		
Modified Geriatric Anxiety Inventory				
ALS (249)	20	194	0.355	0.05
Control (130)	20	183		
FEMALES				
Life Events Inventory				
All ages				
ALS (149)	726	230	0.685	0.02
Control (318)	753	236		
Age 0–20 y				
ALS (149)	92	230	0.652	0.02
Control (318)	107	236		
Age 21–40 y				
ALS (149)	345	226	0.351	0.04
Control (318)	345	238		
Previous 2 y				
ALS (149)	36	218	0.075	0.08
Control (318)	44	241		
Previous 5 y				
ALS (149)	73	211	0.010*	0.12
Control (318)	98	245		
Previous 10 y				

(*Continued*)

Table 3. (Continued)

Respondents (number)	Median	Mean rank	Mann-Whitney *p*	Effect size *r*
ALS (149)	155	212	0.018*	0.11
Control (318)	190	244		
Occupational Stress				
ALS (143)	1.83	234	0.305	0.05
Control (306)	1.78	221		
Connor-Davidson Resilience Scale				
ALS (149)	80	269	<0.001*	0.17
Control (320)	73	219		
Modified Geriatric Anxiety Inventory				
ALS (149)	20	218	0.062	0.09
Control (320)	23	243		

*: $p<0.05$

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Discussion

We found no differences between ALS respondents and controls in exposures to potentially stressful life events. On the contrary, some female ALS subgroups had *fewer* Life Events Inventory scores than controls, findings opposite to those expected if stress were related to ALS. In addition, self-reported stress from occupations was the same in people with ALS and controls. ALS respondents had on average higher resilience than controls, indicating they would be more likely to be able cope better with stressful events. Finally, people with ALS were not more anxious than controls. Our results do not therefore support the hypothesis that psychological stress is a risk factor for developing ALS.

ALS respondents in this study were found to be more resilient than controls when using a validated method of testing resilience, the Connor-Davidson Resilience Scale [23]. Resilience can be defined as a 'measure of successful stress-coping ability' [23] and is thought to arise from a combination of factors that are personality-related, genetic or biological, and social or environmental (eg, having strong social networks) [28]. The characteristic personality profile of resilient individuals is greater conscientiousness, higher extraversion, and lower neuroticism [29], features similar to the personality type that has been described in people with ALS [30]. Extraversion can manifest itself as a greater ability to communicate positive feelings and a greater ability to access social networks, which would result in a person being better able to manage stressors [4]. People with higher conscientiousness and extraversion feel their life stressors are less stressful than others [31], and could be considered to exhibit greater resilience. Some evidence therefore links resilience to personality types in ALS.

The questionnaire we used for this project is an anonymous online international survey looking at a range of risk factors for ALS [17]. The 165 items in the questionnaire include the Holmes and Rahe checklist for potentially stressful life events [3], to date the most commonly used method to measure life event stressors [32]. Life event checklists have, however, been criticised, predominantly on the basis that the actual responses to a broad category, eg, 'serious physical illness or injury' could result in some respondents including minor illnesses but others including only major illnesses such as cancer, a problem of intracategory variability [32]. To overcome this deficiency in checklists, several approaches have been canvassed, the most promising being prospective semi-structured interviews, performed by trained raters who judge the importance of individual events [32,33]. We were unable to gather this type of detailed narrative information since our respondents were anonymous and unable to be

contacted. However, future life event stressor studies in ALS and other neurodegenerative disorders could use this narrative approach, which is now considered be the gold standard for life event studies [33]. These would face challenges, though, such as the considerable costs of employing trained raters over the prolonged period of time it would take to interview 800 people, since 400 respondents per disease and control group are required for statistical robustness [32], and with less common conditions such as ALS patient recruitment can take a number of years. This economic consideration is the main reason interview-based methods have been used so far in only a small minority of life event studies [32]. It would, in addition, be difficult to undertake interviews in the numerous languages used in an international study such as ours, and ethics approvals for such studies would need to be obtained from multiple institutions in each country, so replicating such narrative studies in ALS or other neurodegenerative diseases would be a major undertaking. Some advantages to using checklists for life events do remain: respondents are more likely to be forthcoming about nominating stressful life events in an anonymous checklist than during an interview, and there is no need to account for inter-rater reliability between interviewers.

Increased resilience in people with ALS is of interest given that several genetic influences on resilience have been described [34]. The genes involved include those related to the hypothalamus-pituitary-adrenal axis and to the serotonin transporter, *COMT* that degrades dopamine and noradrenaline, as well as *NPY* and *BDNF*. It would be of interest to see if similar genetic polymorphisms are more frequent in people with ALS. Furthermore, both gene-gene and gene-environment interactions could underlie variabilities in stress responses, which may be worth exploring in ALS. Epigenetic mechanisms related to resilience are found in mice [34], and these epigenetic changes may be of relevance to ALS since ALS-discordant monozygous twins with no DNA genetic differences on whole genome sequencing [35] have be shown to have numerous epigenetic dissimilarities [36].

Our study has some limitations. (1) ALS patients were asked to remember their psychological state *before* they were diagnosed. This is, however, of limited concern in a disease like ALS which usually has a short course (as shown by the median disease duration of 1 year in this study), compared to disorders with long courses such as multiple sclerosis, where patients would need to recall these aspects many years after diagnosis. (2) The Geriatric Anxiety Inventory was developed for use in a geriatric population, but in this study it assessed people aged 40 years and above. However, the content of the Geriatric Anxiety Inventory is generic and could be applied to a person of any age, and our results showing that females exhibited greater anxiety than males are consistent with other studies where the Geriatric Anxiety Inventory has been used in geriatric populations [37]. (3) It is possible that some control respondents could develop ALS later in life. However, ALS is a relatively uncommon disorder, with a lifetime risk of 1:350 for men and 1:400 for women [38]. Since we had 120 male and 282 female controls, only one of our controls in total would be likely to develop ALS during their lifetime, which would not affect the results. (4) Recall bias needs to be considered as a factor in all case-control questionnaire studies. Our ALS respondents did not report more checklist life events than controls, suggesting little recall bias. In addition, similar proportions of both ALS and non-ALS respondents reported self-volunteered significant life events, further indicating that recall bias was unlikely to be a confounding factor in this study.

Conclusion

Our results do not support the concept that psychological stress from significant life events or stressful occupations plays a role in the pathogenesis of ALS. The higher levels of resilience we found in people with ALS may be associated with pre-morbid personality differences that have

been described in this disease, and further investigations looking for shared genetic variants between people with ALS and those reported in resilience may be of value in investigating the pathogenesis of ALS.

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Chapter 6: A comparison of mercury exposure from seafood consumption and dental amalgam fillings in people with and without amyotrophic lateral sclerosis (ALS)

One of my particular interests is environmental epidemiology and the role of chemicals in the development of disease. I therefore evaluated exposures to sources of mercury and their relevance to ALS. The possibility that heavy metals such as mercury may be involved in ALS pathogenesis appears compelling because of the known neurotoxicity of these compounds. Much of the difficulty in ascertaining whether or not these metals contribute to developing ALS lies in accurately assessing exposure to these compounds, both from occupational and incidental sources (for example, the local environment and hobbies). Mercury exposure has been evaluated as a risk factor for ALS before, but studies have been limited in scope and size [1]. One early study found an association between exposure to mercury and ALS [2], as did some more recent studies [3-5]. However, a definitive link between ALS and mercury remains elusive, since a greater number of studies have not shown any relationship between mercury and ALS [6-15].

I looked at the main sources of environmental exposures to mercury, fish consumption and dental amalgam fillings, to see if these exposures were different between ALS and control respondents. These exposures could represent an accumulating source of mercury, since mercury is known to be taken up into the CNS and remain in neurons indefinitely [16].

The results, presented as a publication in this chapter, found that neither consumption of mercury in seafood nor mercury dental fillings were higher in ALS than controls respondents. It was concluded that, if mercury is a risk factor for ALS, there must be additional sources of mercury exposure that are unaccounted for, or that people with ALS have some particular (probably genetic) susceptibility to mercury toxicity.

These results were presented in a poster at the 29th International Symposium on ALS/MND in 2018 (Appendix J).

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Article

A Comparison of Mercury Exposure from Seafood Consumption and Dental Amalgam Fillings in People with and without Amyotrophic Lateral Sclerosis (ALS): An International Online Case-Control Study

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Abstract: Exposures to toxic metals such as mercury have been suggested to be risk factors for amyotrophic lateral sclerosis (ALS). Human intake of mercury commonly occurs via consumption of seafood or from mercury-containing amalgam dental restorations ('mercury fillings'). We therefore compared mercury exposures from these sources in 401 ALS and 452 non-ALS respondents, using an internationally-available online questionnaire that asked respondents how often they ate seafood and what their favourite types of seafoods were. Respondents were also asked to record numbers of current or former mercury fillings. ALS and non-ALS respondents did not differ in their frequency of seafood consumption or in monthly mercury intake from favourite seafoods. Both groups had similar numbers of current, as well as former, mercury fillings. In conclusion, this study found no evidence that mercury exposure from eating seafood, or from mercury dental fillings, was associated with the risk of developing ALS. Therefore, if mercury does play a role in the pathogenesis of ALS, other sources of exposure to mercury in the environment or workplace need to be considered. Alternatively, a susceptibility to mercury toxicity in ALS, such as genetic or epigenetic variations, multiple toxic metal interactions, or selenium deficiency, may be present.

Keywords: amyotrophic lateral sclerosis; ALS; motor neuron disease; mercury; seafood; fish consumption; dental amalgam filling; case-control study; online questionnaire; international study

1. Introduction

Toxic metals, and mercury in particular, have long been suspected to play a part in the pathogenesis of amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND) [1–3]. Mercury can initiate oxygen free radical formation, induce excitotoxicity, reduce DNA, RNA and protein synthesis, cause epigenetic changes, activate autoimmunity, and interact with microtubules [4], all mechanisms that have been implicated in ALS [5]. Electron microscopic studies indicate that mercury binds selectively to intracellular sulfhydryl-rich membranes such as those of mitochondria, the nucleus, the Golgi apparatus, the endoplasmic reticulum and lysosomes [6], organelles whose functions have been reported to be impaired in ALS [5,7]. Systemically-administered mercury is taken up selectively by rodent spinal and brain stem motor neurons [8]. Mercury is also taken up preferentially by human spinal alpha motor neurons [9], spinal interneurons [10], and corticomotoneurons [11], and has been located in human astrocytes and oligodendrocytes [12]. All of these cells appear to play a part in the pathogenesis of ALS [5,13–15].

Environmental mercury remains a strong candidate as a precipitating factor for ALS, particularly if combined with a genetic predisposition to mercury toxicity [3]. However, despite toxic metals having biological plausibility and some epidemiological links with ALS, caution is advised regarding the limitations of methods used to assess human exposure [16]. One such limitation is that blood or cerebrospinal levels of toxic metals may not necessarily reflect the extent of previous exposure [17], since, for example, mercury remains in motor neurons for long periods after it has been removed from other organs [18]. Major sources of human exposure to mercury are via the consumption of seafood, especially of large predatory fish such as shark, swordfish, mackerel and tuna [19], and from mercury-containing 'silver' dental amalgam restorations [20], here termed 'mercury fillings' [21].

There is concern among people with ALS (as judged by online comments) as to whether they should attempt to reduce their mercury intake by having their dental amalgam fillings removed, or whether they should limit their fish intake. Interest has been rekindled in the mercury hypothesis for ALS [22] with a report of increased toenail mercury in people with ALS, possibly related to seafood consumption [23]. In addition, occasional reports have suggested that removing dental amalgam fillings, or chelation therapy, can result in stabilization or recovery from some forms of ALS [24]. We therefore sought to determine whether people with ALS are more likely than controls without ALS to be exposed to higher levels of mercury from these sources, using an online international questionnaire that gathered data on seafood and dental amalgam sources of mercury. Our results suggest that mercury exposure from these sources alone is not likely to be associated with the risk of developing ALS.

2. Methods

2.1. Setting

This case-control study used data collected between January 2015 and September 2017 from a multilingual web-based questionnaire, ALS Quest [25]. Cases were respondents who stated 'Yes, I have been diagnosed with ALS/MND.' Controls were participants who stated 'No, I have not been diagnosed with ALS/MND.'

2.2. Ethics Approval

The project was conducted in accordance with the Declaration of Helsinki, and was approved by the Human Ethics Committee of the Sydney Local Health District, reference number X14-0357. Responses used for the study were those where respondents consented by clicking an 'I consent' button and then submitted their responses.

2.3. Frequency of Seafood Consumption

Participants were asked 'How often do you eat fish or shellfish?' on an increasing 8-point scale of: Never, Less than once a month, Once a month, 2–3 times a month, Once a week, 2–3 times a week, 4–6 times a week, or Daily.

2.4. Favourite Seafoods

Seafood varies in its content of mercury, so participants were asked 'Please list up to three of your favourite fish to eat'. Names of seafood entered in non-English languages were translated into English (unless it was a country-specific indigenous fish). The average mercury content of individual seafoods was based on the USA Food and Drug Administration (FDA) report of mercury levels in commercial fish and shellfish [19]. The mercury content of Australian seafood species not present in the USA FDA report was taken from an Australian report [26].

2.5. Estimation of Monthly Mercury Exposure from Seafood

The average mercury concentration in μg/g of up to three favourite seafoods was multiplied by 227 g (8 ounces, the weight of a typical seafood serving) [27], and then by the frequency of seafood consumption (adjusted to a monthly value), to get an estimate of monthly mercury exposure from seafood in micrograms (Figure 1). Assumptions underlying this estimate were that seafood mercury levels around the world are similar to those in the USA FDA list, and that the frequency of seafood consumption and favoured types of seafood remain reasonably stable over a long period of time. To compare types of seafood eaten, shellfish (e.g., prawn, crab and lobster) and cephalopods (e.g., squid, calamari and octopus) were categorised separately from finfish.

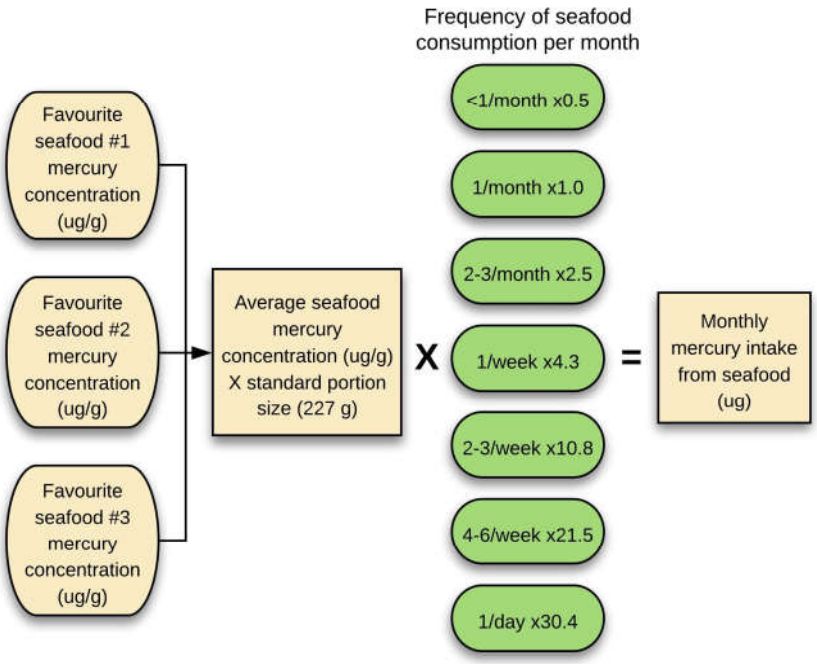

Figure 1. Estimating mercury exposure from seafood consumption. The average mercury concentration in μg/g from up to three favourite seafoods was multiplied by a standard portion size of seafood (227 g), then multiplied by the monthly frequency of seafood consumption to estimate monthly mercury exposure from seafood in micrograms.

2.6. Mercury-Containing Dental Fillings

Respondents were asked 'Have you ever had an amalgam restoration (silver filling) as part of dental care?'. If they responded 'Yes' they were asked: 'How many amalgam silver dental fillings do you currently have? For people with ALS/MND, enter the number you had before being diagnosed. You may need somebody to help you count the silver fillings in your mouth.' Respondents were requested to indicate how many current fillings were occlusal, i.e., 'those that involve the top surface of the tooth (where you bite)' and non-occlusal, i.e., 'those that involve the side of the tooth only'. If they had no current mercury fillings they were asked to enter '0' for the current number of fillings, and then 'If you currently have no silver amalgam dental fillings, how many have you had in the past?' i.e., 'former-only' fillings. Responses were excluded if non-zero entries were made in both the current and former-only mercury filling categories, or if respondents entered 'Yes' to having ever had a filling but did not list any numbers of fillings. Respondents who responded 'No' were assigned a value of 0 for the number of current (combined occlusal and non-occlusal) and former-only fillings.

2.7. Statistical Analyses

Data from the Qualtrics server were transferred to IBM Statistical Package for the Social Sciences (SPSS) for Macintosh (version 22, IBM, Armonk, NY, USA) and GraphPad Prism 7 files. Extreme outliers (more than three times the inter-quartile range) in continuous variables were removed (numbers can be seen in the flow diagram). Nonparametric continuous variables were compared using Mann-Whitney U tests, and normally-distributed continuous variables with *t*-tests. Odds ratios with 95% confidence intervals and Fisher's exact tests were used for categorical variables when all cell numbers were $\geq$5. Significance was assessed at the 0.05 level. No significant male vs. female differences were found in any of the seafood or mercury filling variables (data not shown, see Table S1) so the genders were analysed together.

3. Results

3.1. Cases and Controls

From an initial pool of 1097 questionnaire respondents, 853 eligible ones remained after inclusion criteria (i.e., aged 40 years and over, answered seafood and/or dental questions) were applied (Figure 2).

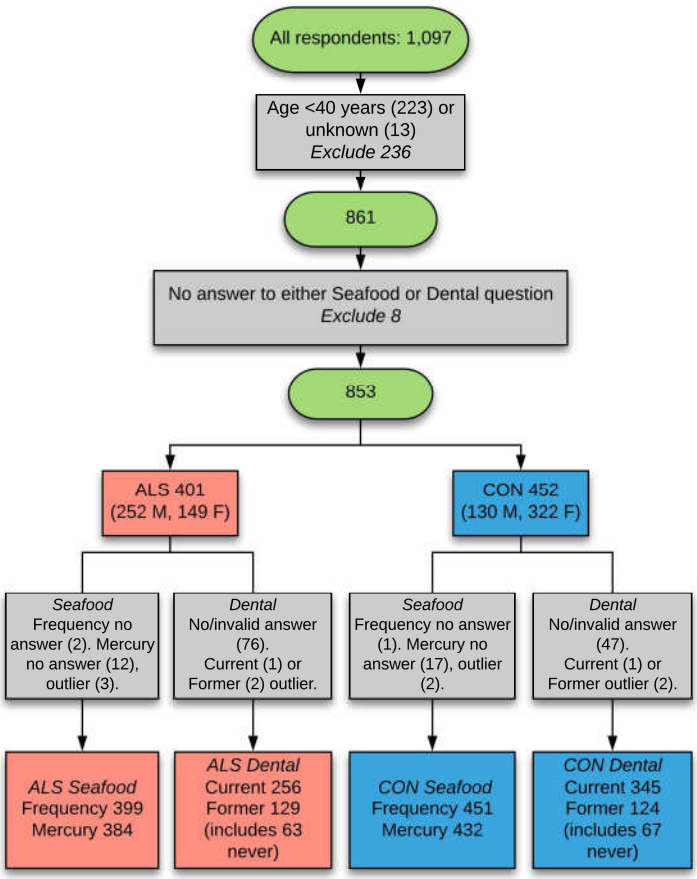

Figure 2. Selection of respondents for analysis. The final datasets of respondents used for analyses was achieved after exclusion criteria were applied for younger age, not answering questions on seafood consumption or dental fillings, and removal of outlier values. CON: control; current: current dental fillings; former: former-only dental fillings; frequency: frequency of seafood consumption; mercury: mercury content of favourite fish; never: replied 'never had any current or former mercury fillings'; outlier: extreme outliers (more than three times the inter-quartile range); M: male; F: Female.

These comprised 401 ALS respondents (252 male, 149 female) and 452 non-ALS controls (130 male, 322 female). The mean age of ALS respondents was 61.5 years (SD 9.2 years, range 40–87 years) and of controls was 57.3 years (SD 10.4 years, range 40–89 years), a significant difference on *t*-testing ($p < 0.001$). When evaluated by gender, the mean ages of male ALS respondents (62.0 years) and male controls (61.8 years) were not significantly different, while female ALS respondents (mean age 60.7 years) were older than female controls (mean age 55.5 years), $p < 0.001$ (see Table S2).

Common sources of information about the questionnaire cited by respondents were: ALS Associations (39%), the Internet (21%), friends (9%), ALS patients (6%), the USA Centers for Disease Control National ALS Registry (5%), health professionals (5%), community groups (4%), Facebook (4%), the Canadian Neuromuscular Disease Registry (2%) and ALS researchers (2%). The composition of the ALS and control groups was similar with regards to country of residence, ancestry and cultural group. The majority of respondents resided in Australia, the USA and Canada, though residents of a further 29 countries supplied responses (Table 1).

Table 1. Demographic characteristics of respondents.

	ALS	***N* (%)**	**Control**	***N* (%)**
Country of residence				
	United States	180 (45%)	Australia	339 (75%)
	Australia	116 (29%)	United States	49 (11%)
	Canada	57 (14%)	Other * (<2% each)	36 (8%)
	Other * (<2% each)	37 (9%)	Spain	14 (3%)
	Spain	9 (2%)	New Zealand	12 (3%)
Ancestry				
	Other (<6% each)	184 (47%)	Other (<4% each)	154 (34%)
	Australian	60 (15%)	Australian	131 (29%)
	English	57 (15%)	English	77 (17%)
	American	37 (9%)	Irish	41 (9%)
	German	32 (8%)	British	25 (6%)
	Irish	23 (6%)	Scottish	19 (4%)
Cultural group				
	American	112 (29%)	Australian	286 (64%)
	Australian	98 (26%)	Other (<2% each)	76 (17%)
	Other (<3% each)	93 (24%)	American	33 (7%)
	Canadian	40 (10%)	English	27 (6%)
	English	28 (7%)	Spanish	14 (3%)
	German	13 (3%)	New Zealander	11 (2%)

Other * (countries of residence): Argentina, Belgium, Brazil, Cape Verde, China, Colombia, Czech Republic, Denmark, Ecuador, Egypt, Finland, Germany, Iran, Ireland, Italy, Luxembourg, Mexico, Netherlands, Portugal, Russia, Slovakia, South Africa, South Korea, Sweden, Switzerland, Turkey, United Kingdom (country not specified).

Eight percent of ALS respondents had at least one relative who had been diagnosed with ALS, and were considered to have familial ALS. The other 92% of ALS respondents were considered to have sporadic (or 'isolated') ALS. As reported by the ALS respondents, 58% had 'classic' (upper and lower motor neuron variant) ALS, 9% progressive muscular atrophy (lower motor neuron variant), 9% progressive bulbar palsy, 8% primary lateral sclerosis (upper motor variant), 8% 'other' and 8% did not know their subtype of ALS. Control respondents were friends (12%), spouses (11%), and blood or non-blood relatives (45%) of ALS patients, individuals from community, research or medical groups (9%), or other categories (22%).

The median online ALS Functional Rating Scale-Revised score [28] (inverted, so that higher scores indicated higher disability) was 13, and scores ranged from 0 to 48 (see Table S1). Most respondents were in the range of 6-18, with decreasing numbers as the scores increased, as expected in ALS [29]. The duration of ALS at the time of completing the questionnaire was calculated by subtracting the

year of diagnosis from the year of consenting to complete the questionnaire. The median duration of disease was 1 year, with the great majority of respondents having disease durations of 4 years or fewer, as expected in ALS [29] (see Table S1).

3.2. Frequency of Seafood Consumption

A similar proportion of ALS (N = 386, 97%) and control (N = 432, 96%) respondents ate seafood. The proportions of respondents who ate seafood at different frequencies, ranging from never to daily, did not differ between ALS and control groups (Figure 3). Chi-square testing of ALS vs. control proportions at each of the eight frequencies showed no statistical differences (data not shown, see Table S1), even at frequencies where there appeared to be slight differences between ALS and controls, i.e., more for ALS at once per month and 2–3 per week, and more for controls at once per week.

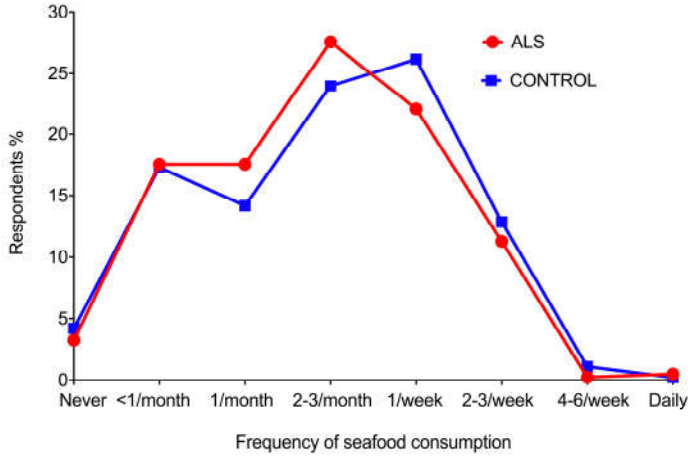

Figure 3. Proportion of respondents eating seafood at different frequencies. ALS and control respondents ate seafood at similar frequencies, ranging from never to daily. None of the slight differences in frequency proportions between ALS and controls was statistically significant.

3.3. Favourite Seafoods

Of the 386 ALS respondents who said they ate seafood, 378 (98%) nominated at least one favourite type, while of the 432 control respondents who said they ate seafood, 417 nominated at least one favourite type (97%) (see Table S1). Some nominated favourite seafoods were not listed in the USA FDA or Australian seafood mercury reports; for the first choice these non-listed seafoods numbered 51 out of 796 cited (6%, 20 ALS and 31 controls), for the second choice 80 out of 737 cited (11%, 27 ALS and 53 controls), and for the third choice 69 out of 636 cited (11%, 27 ALS and 42 controls). A slightly greater proportion of ALS (N = 105, 28%) than control respondents (N = 84, 20%) cited one or more types of shellfish as a favourite type of seafood (OR = 1.5, 95% CI: 1.1–2.1, p = 0.01). No significant difference was found between the 5 ALS (1%) and 14 control (3%) respondents who cited cephalopods as a favourite seafood (OR = 0.4, 95% CI: 0.1–1.1, p = 0.07).

3.4. Mercury Consumption from Favourite Seafoods

No difference between ALS and control respondents was seen in the distribution of monthly mercury exposure from favourite seafoods (Figure 4). The monthly median value for seafood mercury exposure was slightly lower in ALS respondents (39 µg per month for ALS, 49 µg per month for controls) but these values did not differ significantly (p = 0.13) (see Table S1).

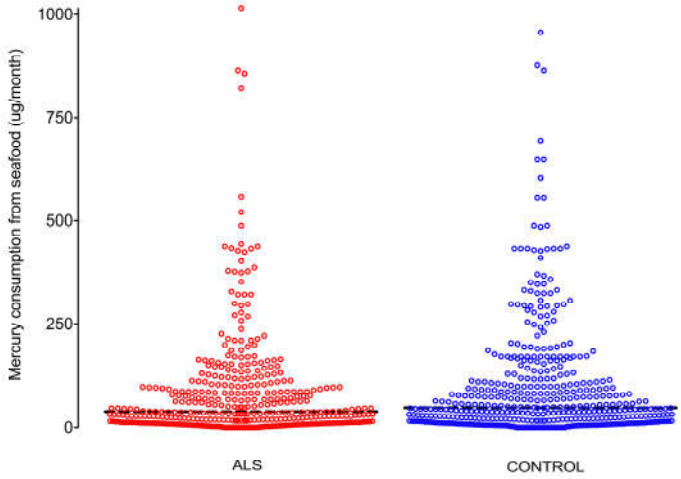

Figure 4. Distribution of mercury exposure from seafood. No difference is seen in the distribution of monthly mercury exposure from seafoods in micrograms between ALS and control respondents. Bar: median monthly exposure (μg).

3.5. Intra- and Inter-Country Comparisons of Mercury Consumption from Favourite Seafoods

The two countries with the largest numbers of respondents were Australia and the USA, so monthly mercury consumption from seafood was compared between, and within, these countries, as well as with all other countries combined (Table 2, and see Table S1). Within all three nationality groups the amount of seafood mercury did not differ between ALS and control respondents. However, both Australian ALS and control respondents had higher seafood mercury consumption than corresponding USA groups. Further analysis showed this was because Australian respondents overall tended to eat seafoods with a higher mercury content (median 54 μg/month) than USA respondents (median 31 μg/month), while the frequency of seafood consumption was similar between these two countries (Australian median about 2–3 times per month and USA median slightly less than 2–3 times per month). For the other combined countries, seafood mercury intake (median 52 μg per month) was similar to that consumed by Australians, whereas the frequency of seafood consumption was higher (at slightly more than 2–3 times per month) than for Australia and the USA.

Table 2. Intra- and inter-country comparisons of monthly mercury consumption from seafood.

	Respondents *N*	**Median Mercury Consumption μg/month**	***p***	**r**
Intra-country: Control vs. ALS				
USA control	47	32	0.80	0.02
USA ALS	177	31		
Australia control	324	51	0.27	0.05
Australia ALS	109	69		
Other countries control	61	65	0.40	0.07
Other countries ALS	98	47		
Inter-country: USA vs. Australia				
USA control	47	32	0.02	0.12
Australia control	324	51		
USA ALS	177	31	<0.001	0.21
Australia ALS	109	69		

N: number, *p*: Mann-Whitney *p* value, r: effect size.

3.6. Mercury-Containing Dental Fillings

The proportions of ALS (N = 262, 81%) and control (N = 338, 83%) respondents who had ever had mercury fillings, i.e., either current (occlusal and non-occlusal) or former-only fillings, were similar (OR = 0.8, 95% CI: 0.6–1.2, p = 0.32), and see Table S1. The distribution of numbers of current fillings did not differ between ALS and control respondents (Figure 5A), with the median number of current fillings being 5 in both ALS and control groups (p = 0.25). ALS respondents (N = 188, 58%) were less likely than controls (N = 264, 65%) to have current occlusal fillings (OR = 0.7, 95% CI: 0.5–1.0, p = 0.04). The distribution of numbers of former-only fillings was similar between ALS and control groups (Figure 5B), with medians of 2 former-only fillings in ALS respondents and 0 in controls (p = 0.46).

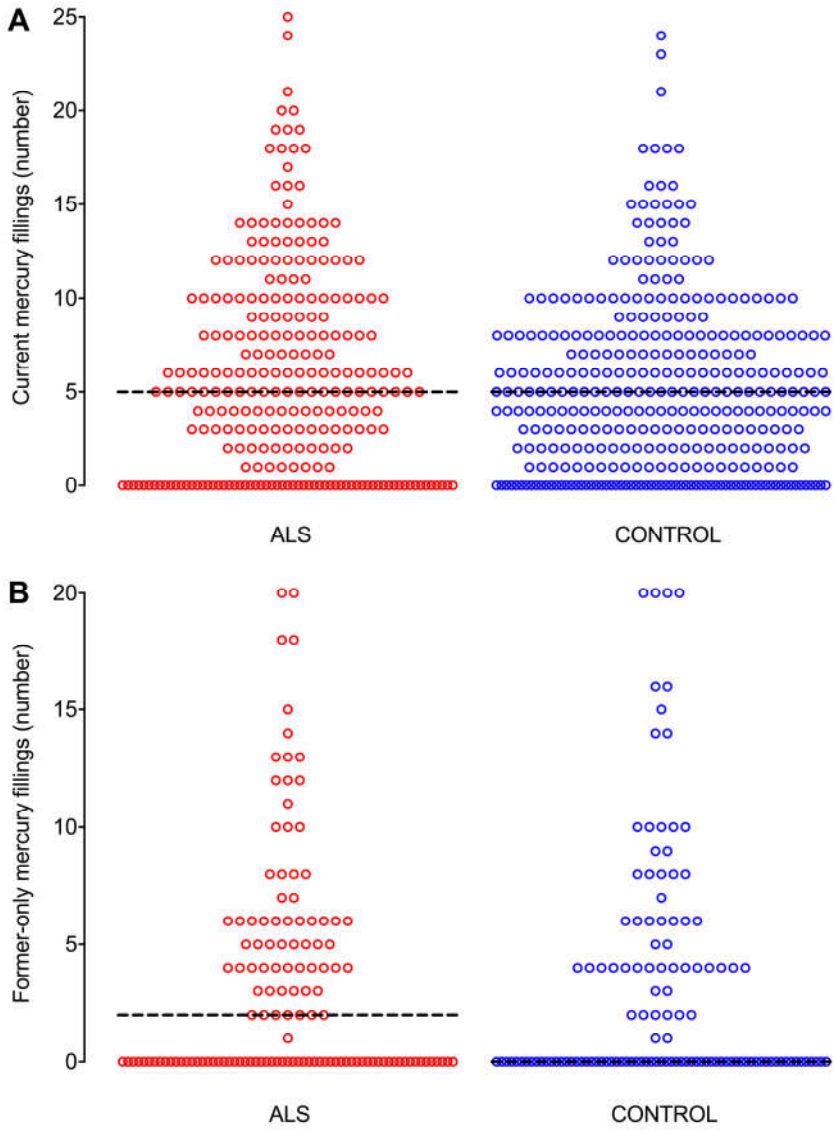

Figure 5. Distribution of numbers of current and former-only mercury fillings. The distribution of numbers of mercury fillings are similar between ALS and control groups for both (**A**) current fillings, and (**B**) former-only fillings. Bar: median number of fillings.

3.7. Intra- and Inter-Country Comparisons of Mercury-Containing Dental Fillings

No differences were seen in median numbers of current (occlusal and non-occlusal) mercury fillings, either between ALS and control respondents who resided within each of the USA, Australia,

and other countries combined, or between USA and Australian ALS and control respondents (Table 3, and see Table S1).

Table 3. Intra- and inter-country comparisons of current mercury amalgam dental fillings.

	Respondents *N*	Median No. of Current Fillings	*p*	r
Intra-country: Control vs. ALS				
USA control	30	4.5	0.49	0.06
USA ALS	103	5.0		
Australia control	270	5.0	0.42	0.04
Australia ALS	84	4.5		
Other countries control	45	5.0	0.76	0.03
Other countries ALS	69	5.0		
Inter-country: USA vs. Australia				
USA control	30	4.5	0.93	0.005
Australia control	270	5.0		
USA ALS	103	5.0	0.80	0.02
Australia ALS	84	4.5		

N: number, *p*: Mann-Whitney *p* value, r: effect size.

4. Discussion

Key findings in this study are that people with and without ALS had comparable intakes of mercury from their favourite seafoods, and had similar numbers of current or former mercury fillings. In fact, ALS respondents were *less* likely than controls to have occlusal mercury fillings, which are more likely to be associated with higher levels of intra-oral air mercury because of chewing [30]. Our study therefore found no convincing evidence that people with ALS are exposed to more environmental mercury than controls from either seafood consumption or from mercury-containing dental fillings.

The U.S. Environmental Protection Agency's acceptable daily dose for methylmercury, i.e., the dose that would not be anticipated to result in any adverse health effects, is 0.1 μg/kg per day [31]. Multiplied by 80 kg (the weight of a typical adult) and 30.8 (the average number of days in a month), this gives a value of 246 μg of mercury per month. The median monthly seafood mercury consumption values for our respondents, 39 μg/month for ALS and 49 μg/month for controls, are well below what the U.S. Environmental Protection Agency considers the maximum acceptable level of exposure for methylmercury, assuming that all the mercury in seafood is present as methylmercury. Therefore, the seafood-related mercury consumption we found in most of our respondents would not be expected to be toxic in the absence of complicating susceptibility factors.

Shellfish, which were more likely to be consumed by our ALS respondents, are low in mercury (0.003–0.100 μg/g) but have been found to contain beta-N-methylamino-L-alanine (BMAA) [32], a postulated risk factor for ALS [33]. This finding therefore supports further research into links between seafood consumption, BMAA and ALS.

The failure to find an increase in mercury exposure from common environmental sources in people with ALS raises the possibility that some susceptibility factors could be responsible for activating mercury that can lie dormant within human motor neurons [9–11]. This susceptibility could be due to genetic polymorphisms, since a number of genetic variants are implicated in susceptibility to mercury toxicity [34], or to acquired epigenetic differences [35,36], both in accordance with the notion that environmental insults, combined with genetic susceptibility and ageing, trigger the common sporadic form of ALS [37]. Of interest in this regard, a gene-environment interaction involving mercury and mutant superoxide dismutase 1 has been described, which results in increased calcium-mediated glutamate excitotoxicity [38]. Mercury is found often in the human nervous system, since almost half of subjects in an autopsy population from different clinicopathological backgrounds have detectable mercury in the brain stem locus ceruleus, a site that appears to be a marker for previous exposure to

mercury [39]. These findings raise the possibility that mercury could be one of the non-genetic factors in a multistep process that is thought to underlie ALS [40]. Susceptibility to mercury in ALS could also arise from multiple toxic metal interactions [41], or from selenium deficiency, since selenium mitigates mercury toxicity [42] and low selenium levels have been implicated in a number of neurodegenerative diseases [43].

Our findings provide no evidence that mercury exposure from eating seafood or from dental fillings is related to ALS, but there remain other sources of mercury exposure that were not assessed in this study. For example, mortality rates from ALS have been found to higher in municipalities of Spain where levels above regulatory limits of heavy metals such as mercury are released into rivers [44]. Mercury is also used in many industrial applications, such as the production of chlorine gas and caustic soda, and is also present in thermometers, barometers, batteries, and electrical switches. If appropriate workplace protections are not in place in these occupational settings, exposure to mercury could occur [45]. Some occupational studies give indirect evidence that workplace exposure to mercury in factories may be implicated in ALS because men with ALS tend to have occupations involving lower skills and tasks, typically associated with factory workers [46]. A recent systematic review of occupational exposures in ALS lists 15 studies in which occupational exposure to toxic metals was reported [47]. In five of these, mercury was analysed as an individual metal [48–52], but case and control numbers were too small in all of these reports for robust statistical analyses.

Another factor to consider, before rejecting the notion of exposure to mercury being related to ALS, is that ocean levels of mercury are less than a quarter of those expected from known mercury emissions such as coal burning, cement production, waste incineration and small-scale gold mining [53]. This has led to the concept of 'missing mercury' [54] and implies that people may be exposed to mercury without ever being aware of its source. These other sources of mercury exposure are not able to be identified using epidemiological methods, so an alternative approach is to look directly for mercury in the tissues of people with ALS. These can be either peripheral tissues, such as toenails [23], or central nervous system tissues where mercury, together with other toxic metals, can now be localised within individual regions and cells using methods such as autometallography [9–11,55] and laser ablation-inductively coupled plasma-mass spectrometry [39].

Of interest is the finding that people in the USA eat seafood with a lower mercury content than do people in Australia (and other combined countries), whereas the frequency of seafood consumption in these two countries is similar. This did not affect our finding of no increased mercury consumption from eating seafood in ALS respondents, but it may have implications for epidemiological studies of mercury-associated disorders when comparisons are made between countries. This finding shows the value of internationally-available surveys in looking at disorders that could be triggered by environmental agents.

The limitations of online questionnaires in the investigation of ALS risk factors have recently been summarised [56]. Limitations specific to the present study are: (1) The female ALS respondents were older than their female controls. However, this means that the ALS females would have had a longer period of time in which to accumulate environmental mercury from the two sources of exposure, but no female ALS-control exposure differences were found to indicate this was the case (data not shown). (2) We were not able to trace the mercury content of a small number of reported favourite seafoods, but the proportion of these was similar in ALS and control respondents. (3) Recall bias is always a concern in case-control studies, but ALS and control respondents gave similar depths of detail in response to questions on seafood consumption and dental fillings, suggesting that recall bias is unlikely to play a major in this study. Furthermore, since we found no differences between ALS and control respondents, any recall bias on the part of control respondents (who could tend to be less assiduous in recalling seafood consumption or numbers of dental fillings) implies that control respondents actually consumed more seafood and had more amalgam filling than they reported, which would reinforce our finding that these exposures are not risk factors for ALS.

5. Conclusions

This international online study did not find evidence that mercury exposure from either seafood consumption or from mercury-containing dental fillings is more common in people with ALS than in controls. If mercury does play a role in the pathogenesis of ALS, it seems likely that other factors such as genetic or epigenetic susceptibilities to mercury toxicity, multiple toxic metal interactions, or selenium deficiency would need to be present to trigger the disease, or that a link between mercury and ALS could arise from other as yet undefined environmental or workplace sources. Further international comparisons of mercury intake in ALS from fish consumption and dental amalgam fillings will be possible once we obtain more responses to our ongoing online risk factor questionnaire.

Supplementary Materials: The following are available online at http://www.mdpi.com/1660-4601/15/12/2874/s1, Table S1 Seafood and dental filling data, Table S2. Ages of respondents.

Author Contributions: J.A.P.K. and R.P.: Concept, methods, investigation, analysis. J.A.P.K.: Writing original draft. R.P.: Data curation, project administration, resources, manuscript review and editing. Both authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Chapter 7: General Discussion and Conclusions

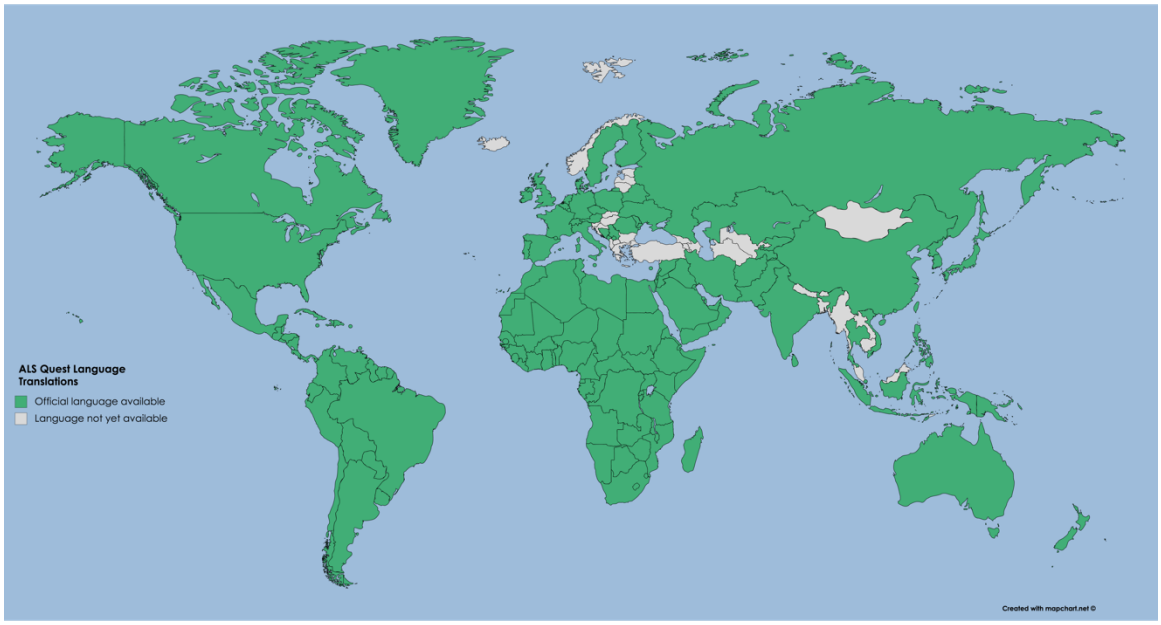
7.1 General Discussion

The goals of this final chapter are to i) bring together the outcomes of my analyses presented in the previous chapters, ii) discuss the implementation and recruitment efforts conducted during the course of this project, and iii) consider future directions of research for the ALS Quest project. In summary, the aims of the project were to develop a comprehensive questionnaire on ALS epidemiology and locate it online, and to evaluate the data obtained from the questionnaire to find ALS risk factors.

A unique feature of this ALS epidemiological study has been the use of an online multi-lingual questionnaire as the basis for collecting data. While the use of a monolingual online questionnaire for ALS has been described in the literature [1], results from that study have yet to be published. This makes ALS Quest the first online questionnaire to be used as the basis for research publications on ALS risk factors. Indeed, the development of the questionnaire and its implementation online encompassed a full year of this project, due to the importance of having a robust and valid research tool. One of the unique features of the ALS Quest questionnaire is the incorporation of material from a variety of other research questionnaires, as well as questions that have not typically been asked of people with ALS, into a single instrument that is available to anyone who can access the internet [2].

A key difference in the ALS Quest questionnaire is its availability in many languages, and the questionnaire includes the first languages used by the great majority of people around the world. ALS Quest is available in 28 languages: Afrikaans, Arabic, Bahasa Indonesia, Chinese, Czech, Danish, Dutch, English, Finnish, French, German, Hebrew, Hindi, Italian, Japanese, Korean, Persian/Farsi, Polish, Portuguese/Brazilian Portuguese, Romanian, Russian, Serbian, Spanish, Swedish, Thai, and Vietnamese. Figures 7-1 illustrates the availability of ALS Quest in the various official/commonly-spoken languages of the world.

Figure 7-1. Map of countries where ALS Quest is available in one or more official languages



The task of translating the questionnaire involved recruiting and managing 32 volunteer translators, which included providing technical assistance, developing instruction manuals to help them with their work, and encouraging them to complete the task. To date, more than 500 questionnaire respondents have worked on ALS Quest in a language other than English, which illustrates the need for a multi-lingual platform in this setting. As a result, I have been able to incorporate data from respondents from more than 30 countries into the analyses, the largest number of individual countries yet included in an ALS epidemiological study.

ALS Quest enabled the collection of data on unproven or unexplored risk factors for ALS. As with any epidemiological investigation, providing negative evidence can be as compelling, and at times more interesting, than positive results. Evaluations of finger ratios and mercury exposure were examples of negative results that add substantially to the discussion of potential ALS causes. In the case of finger ratios, the data obtained from ALS Quest showed that, contrary to a previous report with a limited number of participants [3], ALS and control respondents had similar 2D:4D finger ratios, indicating that prenatal testosterone is not likely to be the link between athletic ability and ALS [4]. This is likely to encourage ALS research community to direct their focus to other reasons why athletic activity appears to be related to ALS, such as head trauma and increased uptake of environmental toxins.

The findings in this project demonstrate that some of the most common sources of mercury in the general population, dental amalgam restorations and seafood consumption, are not to be related to the development of ALS [5]. The practical implications of these findings are that there would be little value in having amalgam fillings removed or limiting consumption of fish in attempts to avoid contracting ALS. Since mercury remains a toxin that could trigger ALS, further efforts could be undertaken to assess mercury exposures from other sources (for example, certain occupations) and to advocate for a reduction in mercury exposures for the human population in general (such as reductions in the burning of fossil fuels). A positive finding from this study was that people with ALS were more likely to consume shellfish, which points towards a possible link between exposure to β-methylamino-L-alanine (BMAA) in shellfish and the development of ALS [6]. Further investigation of this suggested relationship could shed additional light on how BMAA exposure is related to ALS.

Two topics explored by this research project – personality and stress – had either not been evaluated in a rigorous fashion or had not been evaluated quantitatively before. For personality, the anecdotal evidence from clinicians, family members and friends who interact with people with ALS that they are "nice people" [7], but this had not been examined systematically using a comparison of a large group of people with ALS and non-ALS controls. Using the Big Five personality inventory, this project showed that people with ALS do in fact differ from controls in multiple facets of their personality. Both male and female ALS respondents exhibited higher levels of conscientiousness and extraversion than controls, and female ALS respondents had a higher score for agreeableness and a lower score for neuroticism than controls [8].

A recent analysis has characterised the specific personality pattern exhibited by people with ALS as one of four personality types that exist in the general population, referring to it as a "role model" personality type because it is composed of "socially desirable" elements, and linking it to a prior personality type called "resilient" [9]. This suggests that people viewed as socially conforming and resilient may be thought of as "nice" by others. That study, however, did not suggest any hypotheses for how these personality types might arise in particular people.

Another theory regarding personality is the existence of a “General Factor of Personality”, which is approximately equivalent to the “role model” personality type and is referred to as a collection of personality factors related to “social effectiveness” [10]. While the finding that people with ALS exhibit a particular personality pattern is interesting, the question remains as to why a particular personality type would be related to ALS. This leaves open the possibility, as stated in in Chapter 4, that there may be one or more genetic factors in common between personality type and ALS that could underlie both conditions, a topic that could be pursued in future research.

The findings of this personality study have practical implications. For example, these results could help clinicians better understand how to communicate with people with ALS by providing an insight into their personality tendencies. They might also help others consider how to relate to people with ALS, in seeing that some of their actions may originate from a tendency to exhibit “socially desirable” traits, when “social desirability” might not be in their best self-interest. For example, an ALS patient might be dissatisfied with some aspect of their treatment regime but might be inclined to go along with it regardless to avoid being seen as disagreeable.

The results of these ALS personality analyses suggest there may also be aspects that relate to risk factors for ALS. Several examples of these possible connections are described in the Chapter 4 [8], one example being that people who tend to be extraverted (such as those who develop ALS) are more likely to smoke, which is considered to be a risk factor for the disease [11]. This hypothesis suggests that it is not so much that the personality type itself is a risk factor for ALS, rather that the personality type leads to certain behaviours that are themselves risk factors for ALS.

Psychological stress as a possible risk factor for ALS is often mentioned by people with ALS, as indicated by a third of ALS respondents in the ALS Quest study citing it as a possible cause of their ALS (unpublished results). The ALS Quest study, however, did not find that ALS respondents experienced more stress in their lives than controls, either from their life experiences or from their occupations [12]. These findings therefore do not support any links between ALS and stress-induced increased uptake of toxins [13], nor do they do support links

between psychological stress, high oxidative stress, and decreased telomerase activity in the pathogenesis of ALS [14].

ALS respondents displayed a higher level of pre-diagnostic resilience, which implies that they are less likely to have a negative response to stress than others, thus further limiting the likelihood of a connection between stress and ALS. This finding does provide a potential link between resilience and the ALS personality type, which has been characterised as a "resilient" phenotype [9]. Resilience arises from a combination of genetics and environmental and social factors, so there could be a genetic link between personality and resilience [15] that would be worth pursuing further with the large ALS genomic banks now available.

7.2 Implementation of and Recruitment for the ALS Quest Questionnaire

As the sole doctoral researcher working with Dr. Pamphlett on this project, I was the manager of the ALS Quest program and the person primarily responsible for the project. One responsibility was being the public face of ALS Quest for presentations, meetings and social media. As part of our work on finger measurements and the prenatal testosterone hypothesis, I visited eight Rotary groups to give presentations about ALS Quest and to obtain data for a finger measurement validation study (Appendix G). In addition, I travelled to MND Associations in New South Wales, Victoria, South Australia and Western Australia to talk with their staffs and people with ALS about ALS Quest and to encourage their participation. I also participated in an MND NSW Ask the Experts session and multiple scientific symposia.

This project has taught me a multitude of skills that I would not otherwise have learned. Chief among these is the judicious use of social media to convey information about research. For ALS Quest, I have used Facebook, Twitter and YouTube as vehicles to disseminate information about participating in the questionnaire, to let people know about updates such as the addition of a new language translation to the questionnaire, and to publicise the publication of research papers. I also took footage from an interview conducted by MND Victoria staff and created a video for a video-sharing site. These outlets have been helpful for getting the word out about the project and our publications to a wide audience in an efficient manner. A large proportion of our respondents (about 25 percent) reported that the Internet or Facebook was their source for finding out about ALS Quest.

One area of ALS Quest where there were many people involved was in translating the questionnaire. For this task, I managed a team of 32 volunteers working on the various translations of the questionnaire over a course of many months. Many of these were students at the University of Sydney, and I provided them with instructions and any support that was needed. I maintained contact with the translators to encourage them in their task and to ask questions when they arose. This was an integral part of the development of ALS Quest and enabled collection of data from speakers of 25 different languages around the world.

7.3 Directions for Future Research

The ALS Quest questionnaire still has a great deal of information that can be mined from its data set. Additional factors that are being considered for analysis as risk factors are birth weight, medication use, head trauma and other injuries, athletic activity, previous illnesses, family medical history of diseases other than ALS, occupational history, and diesel exposures.

Potential directions for future research by other investigators would be: i) to explore other reasons for a possible link between physical activity and ALS, given the lack of evidence for the prenatal testosterone hypothesis, ii) to further evaluate BMAA exposure from shellfish as a potential contributor to ALS pathogenesis, and iii) to consider potential genetic factors underlying personality and resilience that could have a commonality with ALS genetic factors.

One of the goals of ALS Quest has been to collect a sufficient number of responses so that risk factors can be compared among different countries. This would address a data gap that is persistent in the ALS literature [16]. While some country-specific analyses have been conducted with the data from ALS Quest [5], the number of countries that can be compared at the present time is essentially two (the United States and Australia), so further efforts are needed to continue to recruit ALS Quest respondents from other countries.

7.4 Conclusions

I have developed an online epidemiological questionnaire that provides a comprehensive data set for assessing potential ALS risk factors and is accessible worldwide in many languages. Using data from ALS Quest, I have investigated several ALS risk factors, including some that had not previously been evaluated in a comprehensive way. I have provided

evidence that several hypotheses for ALS risk factors are unlikely to be correct, including high prenatal testosterone, some sources of mercury exposure, and psychological stress. On the other hand, I have found that people with ALS do exhibit a different personality type and are more resilient from people without ALS, which has implications for future genetic studies.

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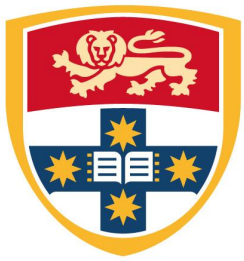
APPENDIX A

Copy of ALS Quest Questionnaire

Note that this electronic PDF version is a complete version that does not demonstrate the skip logic used in the online version of the questionnaire (for example, controls without ALS would not see online questions related to ALS). Therefore, some questions appear to be duplicated, but in the online version these would be selectively displayed depending on how the participants had filled out the questionnaire.

INFORMATION FOR PARTICIPANTS



THE UNIVERSITY OF SYDNEY

ALS Quest: An online questionnaire for research into amyotrophic lateral sclerosis and motor neuron disease

INFORMATION FOR PARTICIPANTS

Amyotrophic lateral sclerosis and motor neuron disease (ALS/MND)

Amyotrophic lateral sclerosis (ALS) and motor neuron disease (MND) are different names for a group of disorders that damage the nerve cells that control muscles, and this leads to progressive weakness.

We are interested in looking at the potential of environmental conditions to play a part in causing ALS/MND. This questionnaire has therefore been set up to gather a wide range of information that can be compared between people who have, and those who do not have, ALS/MND. We hope this information will prove useful in searches for measures that can prevent ALS/MND and for treatments of ALS/MND.

The questionnaire

You will be asked to fill out a questionnaire, which will take approximately 90 minutes to complete.

You can do this in one session or in multiple sessions (there is no limit to the number of times you can enter and leave the questionnaire). However, please note that if you do not access the questionnaire for a period of four weeks, the questionnaire will be terminated and you will not be able to re-access it.

All that is needed to complete the questionnaire is a computer (a PC or MAC) connected to the Web (you cannot use a tablet or a smartphone to complete this questionnaire).

Even if you do not have time to fully complete the questionnaire, we would strongly encourage you to submit whatever answers you have time to complete, as the answers

provided will still be of assistance to the project.

The questionnaire includes a variety of questions about you and your life. Apart from a few preliminary questions at the beginning of the questionnaire, answering all questions is voluntary (so you can choose not to answer a question if you wish).

People who are eligible to complete the questionnaire

Both people with and without ALS/MND are eligible to complete the questionnaire. It is important to have as many participants as possible who do not have ALS/MND also completing the questionnaire.

1. **People who have ALS/MND are eligible to complete the questionnaire if the diagnosis has been made by a neurologist.**
2. **A person with ALS/MND can complete the questionnaire without asking anybody else to do so. However, we encourage people with ALS/MND to ask their spouse/partner and friends (of either gender) to also complete the questionnaire. People with ALS/MND are asked to give their spouse/partner and friends a 6-digit code so that their answers can be compared statistically.**
3. **Any member of the community who has an interest in ALS/MND is encouraged to complete the questionnaire. This includes, for example, individual members of the community, members of community organisations, health workers, and scientists/researchers.**

Minimum age

You need to be aged 18 years or more to complete the questionnaire.

Benefits of the study

While we intend that this questionnaire (and the data that arise from it) furthers medical knowledge and may improve prevention and treatment of ALS/MND in the future, it may not be of direct benefit to you.

Dissemination of research results

The results from the study will be presented at professional conferences and will be published in peer-reviewed scientific journals.

Confidentiality

Since your questionnaire responses are anonymous, no identifiable information about you is obtained for this study. Your questionnaire responses will be transferred to a secure computer database at The University of Sydney, and will then will be removed from the secure Qualtrics server that initially processes the questionnaire (Qualtrics is the name of the software that drives the questionnaire).

Spouse/partners and friends will not be able to use the 6 digit code (given to them by the person with ALS/MND) to access the questionnaire responses of the person with ALS/MND, and vice versa.

The Qualtrics survey software initially collects the IP address (the Internet Protocol that identifies a computer) of the computer from which the questionnaire originated. However, this IP address will be deleted from the Qualtrics server after your response has been transferred to the University of Sydney database, and then also deleted from The University of Sydney database, so no record will be kept of the computer that you used to respond to the questionnaire.

Discontinuing your participation

Even after you have given consent to undertake the questionnaire, you are free to withdraw your information in the questionnaire at any time, without giving any reason, simply by leaving the questionnaire (and without clicking the "Submit" button at the end of the questionnaire). Your non-submitted answers will be deleted by the questionnaire administrator.

If you want to terminate the questionnaire immediately, you can remove your answers by removing Cookies on your computer (see later for how to do this). The questionnaire administrator will delete your non-submitted answers.

After you have clicked the "Submit" button your answers will be transferred to the database and cannot be removed, since we have no way of identifying individuals from whom the submitted questionnaire data have come.

Contacts

The questionnaire is managed by A/Prof Roger Pamphlett at the University of Sydney, Australia. To contact the administrators of the questionnaire for any questions or comments please email als.quest@sydney.edu.au.

This study has been approved by the Ethics Review Committee (RPAH Zone) of the Sydney Local Health District. Any person with concerns or complaints about the conduct of this study should contact the Executive Officer on 02 9515 6766 and quote protocol number X14-0357.

To begin (or continue) the questionnaire, please click the forward (>>) button in the lower right corner of the screen.

GUIDE TO COMPLETING THE QUESTIONNAIRE

GUIDE TO COMPLETING THE QUESTIONNAIRE

Setting Up the Questionnaire

- **You will need a desktop or laptop computer to complete the Questionnaire. *You cannot use a tablet or smartphone to complete the Questionnaire.***
- **This Questionnaire is compatible with the most common Internet browsers such as Firefox, Internet Explorer, Chrome, and Safari. If you are having problems taking the Questionnaire with your current browser, we recommend that you try using the Firefox Internet browser, as this browser has been shown to work best with the Questionnaire. The Firefox browser can be downloaded free of charge from the Web at www.mozilla.com.**
- **Once you have started the Questionnaire, you need to continue using the *same* computer to complete the Questionnaire.**
- **Remember that you may complete the questionnaire in multiple sessions. This gives you time to gather information you may not have to hand.**
- **The Questionnaire is compatible with voice-recognition and speech-to-text programs.**
- **One of the questions asks for measurements, so you may find it useful to have a rigid ruler (not a tape measure) on hand before you begin.**
- **For people with ALS/MND who are unsure about the type of ALS/MND they have (see the**

ALS CLINICAL INFORMATION section for details), we recommend contacting your neurologist or family doctor/general practitioner early for this information, since it may take some time before you receive it.

Viewing the Questionnaire

- **If you'd like to make the text of the Questionnaire larger or smaller on your screen, you can use the zoom function of your Internet browser. In Firefox, you can adjust the zoom under the View menu.**
- **You can also use the keyboard commands CTRL (on a PC) or COMMAND (on a Mac) and + (plus) for zoom in, or CTRL or COMMAND and - (minus) for zoom out.**

Progressing through the Questionnaire

- **Use the Forward (>>) and Back (<<) buttons to progress through the Questionnaire. When you click the Back button (<<) a window saying "Confirm Navigation" will appear. Simply select the "Go Back" box to go back to the previous page.**
- **When you click the “next” button (>>), first check to see that you are viewing the blue “Qualtrics” banner at the top of the page. This will ensure that you are starting at the top of the page.**
- **You may need to scroll from left to right to view the whole width of a question and its answers, depending on the resolution of your browser window.**
- **Use the Table of Contents icon at the top left corner of the page to jump to different headings. (You may need to scroll to the top of the page to see this.)**
- **Do not use the forward and back buttons on your Internet browser while in the Questionnaire. If you do so inadvertently, click through the prompts in your browser to return to the Questionnaire. You will not have lost any answers.**

Answering Questions

- **To find an item in a dropdown list quickly, you can type the first few letters of the item.**
- **If you want to remove a response entered in a dropdown list, select the blank at the top of the list.**
- **When typing in your answers, you don't have to use capitals/upper case (so you can type in all lower case if you prefer).**
- **You can change answers if you want to.**

Starting/Continuing the Questionnaire

- **You can stop and later resume your session whenever you like. Your answers are saved automatically.**
- **Your answers are saved on a "Cookie" (a piece of Internet data), which has been automatically created by your web browser. Do not delete your Cookies during the process of completing the Questionnaire, for example, by using CCleaner or via a setting that automatically deletes all Cookies when you close your browser. If Cookies are deleted before you submit the Questionnaire, you will need to restart the Questionnaire.**
- **Exit the Questionnaire simply by closing the window that you are working in (you will not be asked to log out).**

- **To resume the Questionnaire, re-enter the Web link. The Questionnaire will continue where you left off.**

Additional Questionnaire Respondents

- **You need to submit your Questionnaire before a second person (for example, your partner) can start their Questionnaire on the same computer. Once you have submitted your Questionnaire, delete all Cookies from your Internet browser (do not delete Cookies until you have finished your Questionnaire, since this will delete all your answers) to allow the second person access to a new Questionnaire. If you need instructions on how to delete Cookies, search Google for "How to delete Cookies" and include your browser name.**

Thank you for participating in this research study

PRELIMINARY INFORMATION

PRELIMINARY INFORMATION

To activate the questionnaire, we need you to first answer a few questions on this page, and then consent to taking the questionnaire. Answering any of the other questions in the questionnaire will be optional.

Do you have amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) that has been diagnosed by a neurologist?

- ○ Yes, I have been diagnosed with ALS/MND
- ○ No, I have not been diagnosed with ALS/MND

What is your connection with ALS/MND?

- ○ I am the spouse/partner of someone with ALS/MND.
  (If your partner has passed away from ALS/MND, please select "I have another connection with ALS/MND" below and specify "Partner had ALS/MND".)
- ○ I am a friend of someone with ALS/MND.
- ○ I have a relative or relatives (either present or past) with a diagnosis of ALS/MND.
- ○ I have another connection with ALS/MND (please specify):
- ○ I have no specific connection with ALS/MND.

How did you hear about this questionnaire?

- From a person with ALS/MND
- From an ALS/MND Association (for example, at a meeting or in a newsletter)
- From the Internet
- From a health professional
- From a community group
- From a friend or acquaintance who does not have ALS/MND
- From a newspaper, television, or radio report
- Other (please specify):

For how many years have you been the spouse/partner of someone with ALS/MND? (before they were diagnosed with ALS/MND)

For how many years have you been a friend of someone with ALS/MND? (before they were diagnosed with ALS/MND)

Please enter the unique identifying number of the spouse/partner with ALS/MND who suggested you undertake the questionnaire:

- Unique number:
- I do not have the unique identifying number of my spouse/partner with ALS/MND who suggested I undertake the questionnaire.

Please enter the unique identifying number of the friend with ALS/MND who suggested you undertake the questionnaire:

- Unique number:
- I do not have the unique identifying number of my friend with ALS/MND who suggested I undertake the questionnaire.

Please create a 6-digit code, consisting of 4 letters and 2 numbers, that you can give to your spouse/partner and friends (*not blood relatives*) that you ask to complete the questionnaire (use the same code for both your spouse/partner and your friends). The letters and numbers can be in any order, for example A12B34 (do not use this example for your code). Whether the letters are in upper or lower case does not matter (it is not case-dependent). Do not repeat any letters or numbers.

Please write your code down separately from the questionnaire, so that you can remember it later if necessary.

Your spouse/partner and friends will not be able to use this code to access your questionnaire responses.

What was your age at your last birthday?

What is your gender?

- Male
- Female

CONSENT

CONSENT

Please click on the "I Consent" button below to indicate your consent to enter data into the questionnaire.

By clicking this button, I:

1. Acknowledge that I have read the "Information for Participants" above and agree to participate in this research.

2. Understand that I will not be asked for any personal information that could identify me, so the study is anonymous and strictly confidential.

3. Freely choose to participate in the study and understand that I can withdraw my questionnaire answers at any time until I click the "Submit" button at the end of the questionnaire.

Please enter today's date using the fields below.

Remember, the questionnaire needs to be submitted within 4 weeks of this date, or your response will be discarded. You may want to make a note of this date 4 weeks from now in your diary.

	Day	Month	Year
Date of Consent			

GENERAL INFORMATION

GENERAL INFORMATION

We would like to know some basic information about yourself.

In which country are you currently living?

In which month of the year were you born?

In which country were you born?

Which of the following best describes the place you were born?

- Urban (population greater than 50,000) - Inner City
- Urban (population greater than 50,000) - Suburb
- Regional centre (population less than 50,000)
- Rural (non-farm)
- Rural (farm)

Please estimate the total number of years that you have lived in each the following types of environment. For people with ALS/MND, include only those places where you lived before you were diagnosed with ALS/MND.

	Number of Years
Urban (population greater than 50,000) – Inner city	
Urban (population greater than 50,000) – Suburb	
Regional centre (population less than 50,000)	
Rural (non-farm)	
Rural (farm)	

Were you delivered by Cesarean section (c-section)?

- Yes
- No
- I don't know

Do you have a written record of your birth weight that you can refer to?

- Yes
- No

What was your birth weight according to the written record? Please choose a unit in the first column and enter a number in the second column. If you'd like to enter your weight in pounds and ounces, use the first text box for pounds and the second text box for ounces.

	Unit	Weight in kg or lb Value	Weight in ounces (if necessary) Value
Enter weight here:			

What is your ancestry? Please select either one or two ancestries (if two, select one in each column; if one, leave the second column blank).

	Ancestry 1	Ancestry 2
Select:		

With which cultural groups do you most identify yourself? Please select either one or two groups (if two, select one in each column; if one, leave the second column blank). These can be the same or different from your ancestries.

	Cultural Group 1	Cultural Group 2
Select:		

What is the highest level of education that you have completed?

- Primary (elementary) school
- Secondary (high) school
- Non-university diploma or certificate
- University undergraduate degree (Bachelor)
- University postgraduate degree (Doctoral, Masters)

How many years of education have you had in total? If any of your education was part-time, please estimate the total in full-time equivalent years.

What was the main field of study of the highest qualification you have completed?

What is your present marital status?

- Never married
- Married (registered)
- Separated
- Divorced
- Widowed

Do you have a de facto partner?

- Yes
- No

What language do you usually speak at home? Please note that English is listed first in the drop-down menu.

Do you see yourself as belonging to any particular religion?

- Yes
- No

Apart from such special occasions as weddings, funerals and baptisms, how often do you attend services or meetings connected with your religion?

- Once a week or more
- Once a month or more
- Sometimes, but less often than once per month
- Never or rarely

Please enter the amount of annual total personal income *you* received (before your ALS/MND diagnosis). Please indicate the amount as a whole number (that is, no decimal point, for example, for $75,000 enter 75000). Please note that the Australian dollar and the United States dollar are listed first in the drop-down menu.

	Currency	Amount
Personal Annual Income		

What is *your* personal annual total income? Please indicate the amount as a whole number (that is, no decimal point, for example, for $75,000 enter 75000). Please note that the Australian dollar and the United States dollar are listed first in the drop-down menu.

	Currency	Amount
Personal Annual Income		

Please enter the amount of annual total income *your family* received before your ALS/MND diagnosis. Please indicate the amount as a whole number (that is, no decimal point, for example, for $75,000 enter 75000). Please note that the Australian dollar and the United States dollar are listed first in the drop-down menu.

	Currency	Amount
Family Annual Income		

What is the annual total income of *your family*? Please indicate the amount as a whole

number (that is, no decimal point, for example, for $75,000 enter 75000). Please note that the Australian dollar and the United States dollar are listed first in the drop-down menu.

	Currency	Amount
Family Annual Income		

PHYSICAL FEATURES

PHYSICAL FEATURES

We would appreciate knowing certain information about your physical appearance.

What is your weight? Please enter your usual weight (before your ALS/MND diagnosis).

Choose the unit of measurement in the first column and enter a numerical weight in the second column.

	Unit	Weight Value
Enter weight here:		

PHYSICAL FEATURES

We would appreciate knowing certain information about your physical appearance.

Please enter your current weight.

Choose the unit of measurement in the first column and enter a numerical weight in the second column.

	Unit	Weight Value
Enter weight here:		

What is your height? Choose the unit in the first column and enter the numerical value in the first text box. If you choose to enter your height in feet and inches, use the first text box for feet and the second text box for inches.

	Unit	Height in cm or ft Value	Height in inches (if necessary) Value
Enter height here:			

Are you right- or left-handed?

- Right-handed
- Left-handed
- Ambidextrous

With which foot would you kick a ball?

- Right foot
- Left foot

We would like to know the lengths of your index and ring fingers.

If you cannot straighten your fingers on both hands, please select the option below to skip this question. If you can straighten the fingers on only one hand, do not select this option.

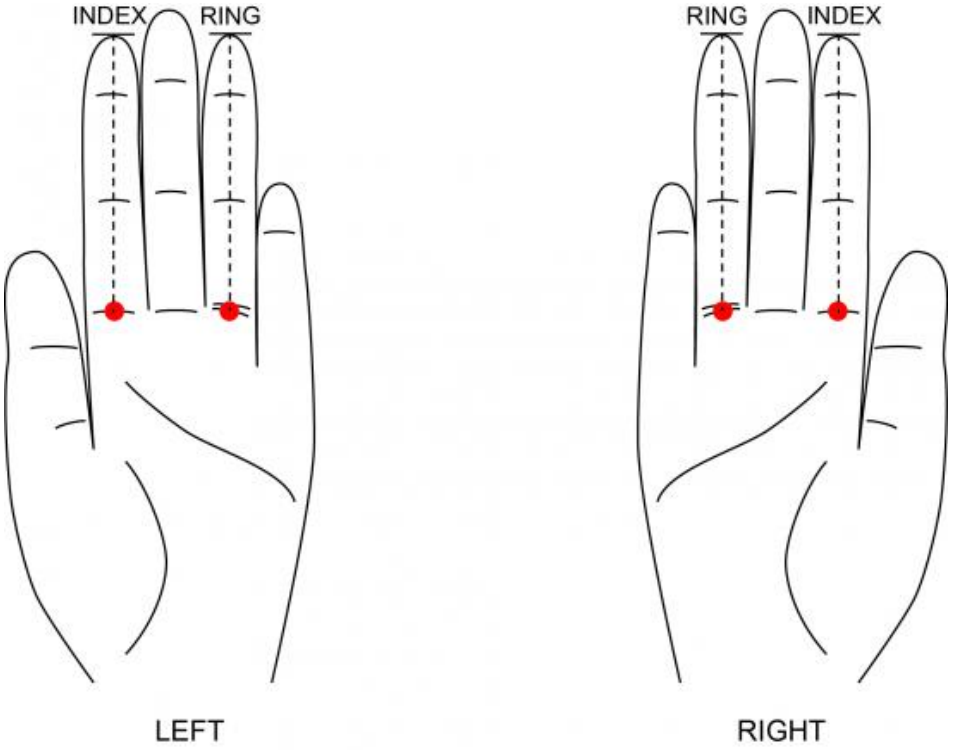
- I cannot straighten out the fingers on both of my hands, when I lay the back of my hands down on a flat surface.

Please measure the lengths of your index (second) and ring (fourth) fingers on each hand. The diagram below shows you how to make the measurements.

If you can only straighten out the fingers on one hand, only record the measurements for that hand.

If possible, please take off any rings prior to measuring your fingers.

First lay the back of your hand out flat on a level surface, with your fingers together. Then, with a rigid millimetre ruler (not a tape measure), measure from (1) the tip of the finger to (2) the middle of the crease at the bottom of the finger. The ring finger often has two of these creases, so here measure from the crease that is closest to the wrist.



	Left Hand (mm)	Right Hand (mm)
What is the length of your *index* (second) finger in millimetres?		
What is the length of your *ring* (fourth) finger in millimetres?		

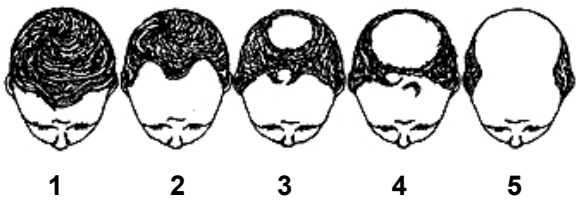
What is your predominant natural hair color? If your hair is now grey, this is the colour of your hair before you turned grey.

- Blonde or Strawberry Blonde
- Brown (light, medium or dark)
- Black
- Red
- Auburn (reddish-brown)

This question is for men aged over 45 years only.

Which of the following most closely approximates your hair pattern at age 45 years?

Please estimate the pattern of hair loss (if any) you had when you were this age with the aid of the figure below. You may need a photograph of yourself at about this help to jog your memory.



- 1. No hair loss
- 2. Mild receding hair line
- 3. Moderate receding hair line and mild hair loss at the top of the head
- 4. Moderate receding hair line and marked hair loss at the top of the head
- 5. Marked hair loss with joining up of receding hair line and hair loss at top of head
- Not applicable (that is, you are less than 45 years old)

What is your predominant natural eye color?

- Brown
- Blue
- Green
- Green-Brown (Hazel)
- Grey

Which colour best describes the colour of the skin <u>on the inside of your upper arm</u>, that is, your skin colour without any tanning?

- Very fair
- Fair
- Medium
- Olive
- Dark
- Very dark
- Black

What would happen to your skin if it was repeatedly exposed to bright sunlight in summer without any protection?

- Go very brown and deeply tan
- Get moderately tan
- Get mildly or occasionally tan
- Get no suntan at all or only get freckled

OCCUPATIONS

OCCUPATIONS

Next are some questions about your occupational history. Please provide as detailed a response as possible (you are not limited in the length of your response by the size of the boxes). This may take several minutes, but it is important information.

Please provide your occupational history (including part-time employment) from your first job (top row) up to the present day, for jobs you held for 6 months or longer. Please only list your occupations up until the time you were diagnosed with ALS/MND.

If you stayed with the same employer but changed the type of work you did (for example, you changed from Grocery clerk to Assistant manager) please consider that as two occupations, even though they were for the same employer. If you went back to a previous occupation after a break, enter it a second time.

1. In the first column, "Occupation Title", give the full title of the occupation, for example, Childcare aide, Maths teacher, Pastry cook, Tanning machine operator, Apprentice toolmaker, or Sheep and wheat farmer. For public servants provide the official designation and occupation. For armed services personnel provide rank and occupation (note: there is an additional section for Armed Forces personnel). For all unpaid work in the home, use the Occupation Title "Domestic duties", Employer Business "Various", and Duties "Various".

2. In the second column, "Employer Business" describe the industry or business of the Employer, for example, Repairs and maintenance, Education, Agriculture, Finance, Medicine, or Telecommunications service.

3. In the third column, "Duties", describe what activities you performed in this occupation.

4. In the next column please enter the number of years you spent in that occupation.

5. In the final three columns, please estimate the level of physical activity, the level of stress involved, and the amount of time outdoors for each occupation.

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3. In the third column, "Duties", describe what activities you performed in this occupation.

4. In the next column please enter the number of years you spent in that occupation.

5. In the final three columns, please estimate the level of physical activity, the level of stress involved, and the amount of time outdoors for each occupation.

Please enter your occupations in this table, with your first job in the first line:

	Occupational History			Number of Years	Physical Activity Involved	
	Occupation Title	Employer Business	Your Duties			
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Have you ever served in the military or armed services?

- Yes
- No

Which branch of the military or armed services did you serve in?

- Army
- Navy
- Air Force
- Other (please specify):

Have you ever been deployed on active duty?

- Yes
- No

For each deployment, please select your age at the time of deployment, enter the location where were you deployed, and specify what your role in the deployment was (for example, air combat, cook, transport, administration). If you have been on more than 10 deployments, please try to list those deployments that were longest in duration.

	Age	Location	Role
Deployment 1			
Deployment 2			
Deployment 3			
Deployment 4			
Deployment 5			
Deployment 6			
Deployment 7			
Deployment 8			
Deployment 9			
Deployment 10			

Have you had exposure to chemicals in your workplace?

- Yes
- No

Have you been exposed to any of the following chemicals? Please answer to the best of your knowledge, and check off any that may apply.

Under "Other" (at the end of the list), you can enter chemical, trade, and/or common names if you do not see the relevant chemical in the list.

- [ ] Acetylaldehyde
- [ ] Acetone
- [ ] Aliphatic chlorinated hydrocarbons
- [ ] Aliphatic hydrocarbons
- [ ] Amyl acetate
- [ ] Aromatic hydrocarbons
- [ ] Benzene
- [ ] Benzidine
- [ ] Carbon disulphide
- [ ] Carbon tetrachloride
- [ ] Cyclohexane
- [ ] 1,2-Dichloroethane (synonym: ethylene dichloride)
- [ ] Dichloromethane (synonym: methylene chloride)
- [ ] N, n-dimethylformamide
- [ ] 1,4-Dioxane
- [ ] Epichlorhydrin
- [ ] Ethyl acetate
- [ ] Ethanol
- [ ] Ethyl ester
- [ ] Ethylene/propylene glycol
- [ ] 2-Ethoxyethanol (acetate)
- [ ] Formaldehyde (synonym: formalin)
- [ ] Glycol ethers
- [ ] Heptane
- [ ] Hexane
- [ ] Methanol (synonym: wood alcohol)
- [ ] Methyl n-butyl ketone (synonyms: MBK, 2-hexanone)
- [ ] Methyl ethyl ketone (synonyms: MEK, 2-butanone)
- [ ] Methyl tert butyl ether (MTBE)
- [ ] Methylene chloride (synonym: chloroform)
- [ ] 2-Methoxyethanol (acetate)
- [ ] Naptha
- [ ] Perchloroethylene/Tetrachloroethylene/PCE
- [ ] Stoddard solvent
- [ ] Styrene
- [ ] Toluene
- [ ] 1,1,1-Trichloroethane (synonym: methyl chloroform)
- [ ] Trichloroethylene/TCE
- [ ] Trichlorotrifluoroethane (CFC-113)
- [ ] Xylenes (synonym: dimethylbenzene)
- [ ] Other (please specify):
- [ ] Other (please specify):

As part of your occupation, have you ever worked with any of the following? Please check any that apply. If none, select "None of the above" in the last row.

- [ ] Lead
- [ ] Mercury
- [ ] Cadmium
- [ ] Copper
- [ ] Other metal/mineral (please specify):
- [ ] Other metal/mineral (please specify):
- [ ] Other metal/mineral (please specify):
- [ ] None of the above

Either as part of your occupation, or domestically (for example, during gardening) have you ever been exposed to herbicides or pesticides regularly (once or more per week) for a period of 6 months or more?

- ○ Yes
- ○ No

Which herbicides or pesticides have you been exposed to regularly for 6 months or more? List the trade names of the products, and select whether exposure was occupational or domestic.

	Herbicide/Pesticide Name	Exposure Type	
		Occupational	Domestic
Herbicide/Pesticide 1			
Herbicide/Pesticide 2			
Herbicide/Pesticide 3			
Herbicide/Pesticide 4			
Herbicide/Pesticide 5			
Herbicide/Pesticide 6			
Herbicide/Pesticide 7			
Herbicide/Pesticide 8			
Herbicide/Pesticide 9			
Herbicide/Pesticide 10			
Herbicide/Pesticide 11			
Herbicide/Pesticide 12			
Herbicide/Pesticide 13			
Herbicide/Pesticide 14			
Herbicide/Pesticide 15			

Have you ever been exposed to diesel fuel or fumes on a regular basis (at least once a week for 6 months or more), in any of the ways listed below? Please select all that apply.

- [ ] Living near a major road or highway
- [ ] Living in an inner-city
- [ ] Commuting to and from work for a daily total of 2 hours or more
- [ ] Driving a diesel-fueled passenger vehicle
- [ ] Driving a diesel-fueled light commercial vehicle
- [ ] Driving a bus
- [ ] Driving a truck
- [ ] Driving a land, sea or air military vehicle
- [ ] Using diesel-fueled farm equipment
- [ ] Working on a diesel-fueled boat or ship
- [ ] Operating heavy machinery
- [ ] Working on or at a railroad, mine, toll-booth, dock or garage/petrol station
- [ ] Traveling on a school bus as a child
- [ ] Cooking on a diesel-fueled stove
- [ ] Using a diesel-fueled heater
- [ ] Other (please specify):
- [ ] Other (please specify):
- [ ] Other (please specify):
- [ ] None of the above

Have you ever been exposed to a severe electrical shock, that is, a shock that caused a burn or for you to be thrown off your feet?

Only answer "Yes" if the shock occurred *before* you were diagnosed with ALS/MND.

- ○ Yes
- ○ No

Have you ever been exposed to a severe electrical shock, that is, a shock that caused a burn or for you to be thrown off your feet?

- Yes
- No

What age or ages were you when you were exposed to the electric shock? Please list up to three, as applicable.

	Age
First electric shock	
Second electric shock	
Third electric shock	

Did you get your first weakness in the same limb which received the electric shock?

- Yes
- No

PERSONAL HABITS AND PASTIMES

PERSONAL HABITS AND PASTIMES

Do you have any particular dietary practices that you have used for most of your life? Please select any that apply, and indicate only those that you used before your ALS/MND diagnosis.

- [ ] Organic
- [ ] Vegetarian
- [ ] Vegan
- [ ] Lactose intolerant/lactose free
- [ ] Gluten free (or diet suitable for those with celiac disease)
- [ ] Other (please specify):
- [ ] Other (please specify):
- [ ] Other (please specify):

PERSONAL HABITS AND PASTIMES

Do you have any particular dietary practices that you have used for most of your life? Please select any that apply.

- [ ] Organic
- [ ] Vegetarian
- [ ] Vegan
- [ ] Lactose intolerant/lactose free
- [ ] Gluten free (or diet suitable for those with celiac disease)
- [ ] Other (please specify):
- [ ] Other (please specify):
- [ ] Other (please specify):

How often do you eat fish or shellfish?

- ○ Never
- ○ Less than once a month
- ○ Once a month
- ○ 2-3 times a month
- ○ Once a week
- ○ 2-3 times a week
- ○ 4-6 times a week
- ○ Daily

Please list up to three of your favourite fish to eat.

Fish 1

Fish 2

Fish 3

What type of water do you drink on a frequent basis (that is, on most days)? Select any that apply.

- Tap/municipal water
- Well water
- Water from a drilled source (bore water)
- Rainwater
- Water collected and stored in a tank
- Flat bottled water
- Sparkling bottled water

Caffeine consumption

How often do you have a drink containing caffeine?

How many drinks containing caffeine do you have on a typical day?

How often do you have five or more caffeinated drinks on one occasion?

Alcohol Consumption

How often do you have a drink containing alcohol?

How many drinks containing alcohol do you have on a typical day when you are drinking?

How often do you have five or more alcoholic drinks on one occasion?

Have you even been a cigarette, cigar, or pipe smoker?

- Yes
- No

Which tobacco products do/did you smoke at least once per week for six months or longer? Please select any that apply.

- Cigarette
- Cigar
- Pipe

Please answer the following questions about your tobacco usage. If not applicable, choose "Not Applicable" from the dropdown menu.

	Age/Number
What age did you start?	
What age did you stop? (if applicable)	
How many cigarettes per **day** do/did you smoke?	
How many cigars per **week** do/did you smoke?	
How many pipes per **week** do/did you smoke?	

Please check off the boxes below for hobbies that you have participated in, at least one time per month for six months or more.

If none, select "None of the above."

- [ ] Removing old paint from furniture
- [ ] Painting pictures or furniture with oil based paint
- [ ] Home remodeling/renovation projects that involved scraping, stripping, burning or sanding paint. Please list the year that you remodeled/renovated your home:
- [ ] Glazing pottery or other ceramics
- [ ] Working with leaded crystal glass
- [ ] Stained glass or art glass using lead joints
- [ ] Repairing or restoring cars, other than fixing a flat tire or changing oil
- [ ] Building wooden or plastic models using glue
- [ ] Developing photographs
- [ ] Leather work
- [ ] Metal work
- [ ] Soldering
- [ ] Woodworking
- [ ] Silver jewelry work
- [ ] Polishing or grinding gemstones using lead
- [ ] Enameling
- [ ] Gardening or lawn care
- [ ] Outdoor hunting or shooting
- [ ] Gun shooting in an indoor pistol or rifle range
- [ ] Casting of bullets or reloading of ammunition
- [ ] Fishing using lead weights or sinkers
- [ ] None of the above

Please list any other hobbies or pastimes that you have, if not listed above: (if none, enter "None" in the first box)

Hobby 1
Hobby 2
Hobby 3
Hobby 4
Hobby 5
Hobby 6
Hobby 7
Hobby 8
Hobby 9
Hobby 10

Please estimate how many countries (outside of the one you are residing in) you have traveled to in your lifetime (for either holidays or business).

- 0 (that is, you have never left your country of residence)
- 1-10
- 11-20
- 21-30
- 31-40
- 41-50
- More than 50

Over the past ten years, how many airline flights (of any length) have you taken, on average, per year?

Please list any pets that you have had. First list the type of pet (for example, cat or dog), with each pet type in a different line. Then select the total number of each kind of pet, and indicate

whether the pets spent most of their time indoors or outdoors. If you have never had any pets, enter "None" in the top left box only.

	Type of Pet	Total Number	Indoor/Outdoor	
			Mostly indoors	Mostly outdoors
Pet 1				
Pet 2				
Pet 3				
Pet 4				
Pet 5				
Pet 6				
Pet 7				
Pet 8				
Pet 9				
Pet 10				
Pet 11				
Pet 12				
Pet 13				
Pet 14				
Pet 15				

How often do you go boating or swimming in a natural freshwater body, for example in a lake or river (not in the ocean or in a swimming pool)?

- Never
- Less than once a month
- Once a month
- 2-3 times a month
- Once a week
- 2-3 times a week
- Daily

Do you regularly (once a week or more) use an underarm deodorant that contains aluminium (also known as aluminum)?

You can see if your deodorant contains aluminium by checking the list of contents on the side of the container.

- Yes
- No
- I don't know

Have you ever used any medications containing bismuth? (for example, some medications for digestive complaints contain bismuth – you can see this from the list of ingredients on the container)

- Yes
- No
- I don't know

EXERCISE

EXERCISE

Next are a few questions about your participation in sports and physical activities.

Are you, or have you been, physically active and/or participated in organised sports?

- Yes
- No

Please list the physical and/or athletic activities you have participated in.

Please categorise the activity as either leisure activity (that is, activity in your own time), recreational organised sport (that is, a competitive unpaid sport), or professional sport (that is, paid sport).

If you have participated in more than 15 activities, please list the ones you participated in for the longest.

	Type of Activity	Description of Activity	Number of Years Participated	Hours per week (estimate)	Intens Acti
Activity 1					
Activity 2					
Activity 3					
Activity 4					
Activity 5					
Activity 6					
Activity 7					
Activity 8					
Activity 9					
Activity 10					
Activity 11					
Activity 12					
Activity 13					
Activity 14					
Activity 15					

How often do you participate in recreational exercise (for example, going to the gym)?

- Never
- Less than once a month
- Once a month
- 2-3 times a month
- Once a week
- 2-3 times a week
- 4-6 times a week
- Daily

Have you ever engaged in sport that required strenuous physical effort (for example, running a marathon)?

- Yes
- No

Please list the strenuous physical activities that you have participated in.

	Strenuous Physical Activity	Number of Years Participated
Activity 1		
Activity 2		
Activity 3		
Activity 4		
Activity 5		
Activity 6		
Activity 7		
Activity 8		
Activity 9		
Activity 10		
Activity 11		
Activity 12		
Activity 13		
Activity 14		
Activity 15		

MEDICAL AND SURGICAL HISTORY

MEDICAL AND SURGICAL HISTORY

Next are some questions about your medical history. Please provide as much detail as possible.

Please list any conditions for which you have been treated *medically* (as opposed to surgically), and your age at diagnosis. If you have more than 15 conditions, please list those that are/were most significant.

If none, please enter "None" in the top left "Condition 1" box only.

	Medically-Treated Condition	Age at Diagnosis
Condition 1		
Condition 2		
Condition 3		
Condition 4		
Condition 5		
Condition 6		
Condition 7		
Condition 8		
Condition 9		
Condition 10		
Condition 11		
Condition 12		
Condition 13		
Condition 14		
Condition 15		

Have you ever had poliomyelitis (also called polio or infantile paralysis)?

- Yes
- No

At what age did you get poliomyelitis?

Did you suffer any permanent muscle weakness at the time of getting poliomyelitis?

- Yes
- No

Did this muscle weakness increase later when you were an adult?

- Yes
- No

List any conditions for which you have been treated *surgically*, and your age at diagnosis. If you have more than 15 conditions, please list those that are/were most significant.

If none, please enter "None" in the top left "Condition 1" box only.

	Surgically-Treated Condition	Age at Diagnosis
Condition 1		
Condition 2		
Condition 3		
Condition 4		
Condition 5		
Condition 6		
Condition 7		
Condition 8		
Condition 9		
Condition 10		
Condition 11		
Condition 12		
Condition 13		
Condition 14		
Condition 15		

Have you ever had any metallic objects, such as joint replacements or missile fragments, placed or lodged in your body?

- Yes
- No
- I don't know

What type of metallic object was it, and between what ages did you have it? List up to three objects, as applicable.

	Type of metallic object	Age in	Age out (if applicable)
Object 1			
Object 2			
Object 3			

Have you ever experienced any instances where you hit your head or neck, resulting in one or more of the following symptoms?

- **Being dazed, confused, disoriented or “seeing stars” at the time of the incident**
- **Not remembering the injury**
- **Experiencing headache, dizziness, nausea, irritability, or memory impairments following the incident**
- **Losing consciousness**

If you have experienced any of these occurrences, please select from the dropdown menus the age when the trauma occurred, the description of your symptoms, and the circumstances under which the trauma occurred.

	Age of Occurrence	Description of Symptoms	Circumstances of Traum
Trauma 1			
Trauma 2			
Trauma 3			
Trauma 4			
Trauma 5			
Trauma 6			
Trauma 7			
Trauma 8			
Trauma 9			
Trauma 10			
Trauma 11			
Trauma 12			
Trauma 13			
Trauma 14			
Trauma 15			

Please list the times you have had non-head injuries (for example, bone fractures or bruises) severe enough to require medical attention. Record the nature of the injury, the part of the body injured, and the age you were at the time of the injury.

If you have not had any such injuries, please enter "None" in the top left “Injury 1” box.

	Part of body injured	Nature of injury	Age at time of injury
Injury 1			
Injury 2			
Injury 3			
Injury 4			
Injury 5			
Injury 6			
Injury 7			
Injury 8			
Injury 9			
Injury 10			
Injury 11			
Injury 12			
Injury 13			
Injury 14			
Injury 15			

Have you ever had a psychological or psychiatric condition for which you consulted a health professional?

- Yes
- No

Please list the psychological or psychiatric conditions below (up to four), and for each, indicate your age at the initial consultation and what type of health professional you consulted for the condition.

	Condition	Age at Initial Consultation	Health Professional Consulted
Condition 1			
Condition 2			
Condition 3			
Condition 4			

List any medications that you have taken regularly (that is, daily for at least 6 months) during your lifetime.

If none, please enter "None" in the top Medication 1 box only.

	Name of Medication
Medication 1	
Medication 2	
Medication 3	
Medication 4	
Medication 5	
Medication 6	
Medication 7	
Medication 8	
Medication 9	
Medication 10	
Medication 11	
Medication 12	
Medication 13	
Medication 14	
Medication 15	

Have you ever used any vitamin or mineral supplements that contain chromium? The contents of any vitamin or mineral supplements you use can be found on the container.

- Yes
- No
- I don't know

Do you have any allergies?

- Yes
- No

Please list the substances that you are allergic to:

Allergen 1	
Allergen 2	
Allergen 3	
Allergen 4	
Allergen 5	
Allergen 6	
Allergen 7	
Allergen 8	
Allergen 9	
Allergen 10	

What is your blood type?

DENTAL HISTORY

DENTAL HISTORY

Have you ever had an amalgam restoration ("silver" filling) as part of dental care?

- ○ Yes
- ○ No

How many amalgam silver dental fillings do you currently have? For people with ALS/MND, enter the number you had *before* being diagnosed. You may need somebody to help you count the silver fillings in your mouth.

"Occlusal" fillings are those that involve the top surface of the tooth (where you bite). "Non-occlusal" fillings are those that involve the side of the tooth only.

If you do not currently have any silver fillings, please choose "0".

	Number of fillings
Occlusal silver fillings	
Non-occlusal silver fillings	

If you currently have no silver amalgam dental fillings, how many have you had any in the past?

Have you ever had a gold dental filling (restoration)?

- Yes
- No

How many amalgam gold dental fillings do you currently have? For people with ALS/MND, enter the number you had *before* being diagnosed. You may need somebody to help you count the gold fillings in your mouth.

“Occlusal” fillings are those that involve the top surface of the tooth (where you bite). “Non-occlusal” fillings are those that involve the side of the tooth only.

If you do not currently have any gold fillings, please choose "0".

	Number of fillings
Occlusal gold fillings	
Non-occlusal gold fillings	

If you currently have no gold amalgam dental fillings, how many have you had any in the past?

PERSONALITY

PERSONALITY

Next we ask you to complete some questions to help assess your personality.

Below is a collection of phrases that can describe some aspects of a person's personality. For each item, select the option that best indicates how much you agree with the statements as they apply to you. Please select the option that would have applied to you *before* your ALS/MND diagnosis.

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I am able to adapt when changes occur.	○	○	○	○	○
I have at least one close and secure relationship that helps me when I am stressed.	○	○	○	○	○
When there are no clear solutions to my problems, sometimes fate or God can help.	○	○	○	○	○
I can deal with whatever comes my way.	○	○	○	○	○
Past successes give me confidence in dealing with new challenges and difficulties.	○	○	○	○	○
I try to see the humorous side of things when I am faced with problems.	○	○	○	○	○
Having to cope with stress can make me stronger.	○	○	○	○	○

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I tend to bounce back after illness, injury or other hardships.	○	○	○	○	○
Good or bad, I believe that most things happen for a reason.	○	○	○	○	○
I give my best effort no matter what the outcome may be.	○	○	○	○	○
I believe I can achieve my goals, even if there are obstacles.	○	○	○	○	○
Even when things look hopeless, I don't give up.	○	○	○	○	○
During times of stress/crisis, I know where to turn for help.	○	○	○	○	○
Under pressure, I stay focused and think clearly.	○	○	○	○	○

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I prefer to take the lead in solving problems rather than letting others make all the decisions.	○	○	○	○	○
I am not easily discouraged by failure.	○	○	○	○	○
I think of myself as a strong person when dealing with life's challenges and difficulties.	○	○	○	○	○
I can make unpopular or difficult decisions that affect other people, if it is necessary.	○	○	○	○	○
I am able to handle unpleasant or painful feelings like sadness, fear, and anger.	○	○	○	○	○
In dealing with life's problems, sometimes you have to act on a hunch without knowing why.	○	○	○	○	○
I have a strong sense of purpose in life.	○	○	○	○	○

Not true at Sometimes True nearly

PERSONALITY

Next we ask you to complete some questions to help assess your personality.

Below is a collection of phrases that can describe some aspects of a person's personality. For each item, select the option that best indicates how much you agree with the statements as they apply to you.

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I am able to adapt when changes occur.	○	○	○	○	○
I have at least one close and secure relationship that helps me when I am stressed.	○	○	○	○	○
When there are no clear solutions to my problems, sometimes fate or God can help.	○	○	○	○	○
I can deal with whatever comes my way.	○	○	○	○	○
Past successes give me confidence in dealing with new challenges and difficulties.	○	○	○	○	○
I try to see the humorous side of things when I am faced with problems.	○	○	○	○	○
Having to cope with stress can make me stronger.	○	○	○	○	○

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I tend to bounce back after illness, injury or other hardships.	○	○	○	○	○
Good or bad, I believe that most things happen for a reason.	○	○	○	○	○
I give my best effort no matter what the outcome may be.	○	○	○	○	○
I believe I can achieve my goals, even if there are obstacles.	○	○	○	○	○
Even when things look hopeless, I don't give up.	○	○	○	○	○
During times of stress/crisis, I know where to turn for help.	○	○	○	○	○
Under pressure, I stay focused and think clearly.	○	○	○	○	○

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time
I prefer to take the lead in solving problems rather than letting others make all the decisions.	○	○	○	○	○
I am not easily discouraged by failure.	○	○	○	○	○
I think of myself as a strong person when dealing with life's challenges and difficulties.	○	○	○	○	○
I can make unpopular or difficult decisions that affect other people, if it is necessary.	○	○	○	○	○
I am able to handle unpleasant or painful feelings like sadness, fear, and anger.	○	○	○	○	○
In dealing with life's problems, sometimes you have to act on a hunch without knowing why.	○	○	○	○	○
I have a strong sense of purpose in life.	○	○	○	○	○

	Not true at all	Rarely true	Sometimes true	Often true	True nearly all the time

Below is a second set of statements about personality. For each item, please select the option that best indicates how much you agree with the following statements as they apply to you. Please select the option that would have applied to you *before* your ALS/MND diagnosis.

I am someone who...

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is talkative	○	○	○	○	○
Tends to find fault with others	○	○	○	○	○
Does a thorough job	○	○	○	○	○
Is depressed, blue	○	○	○	○	○
Is original, comes up with new ideas	○	○	○	○	○
Is reserved	○	○	○	○	○
Is helpful and unselfish with others	○	○	○	○	○
Can be somewhat careless	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is relaxed, handles stress well	○	○	○	○	○
Is curious about many different things	○	○	○	○	○
Is full of energy	○	○	○	○	○
Starts quarrels with others	○	○	○	○	○
Is a reliable worker	○	○	○	○	○
Can be tense	○	○	○	○	○
Is ingenious, a deep thinker	○	○	○	○	○
Generates a lot of enthusiasm	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Has a forgiving nature	○	○	○	○	○
Tends to be disorganized	○	○	○	○	○
Worries a lot	○	○	○	○	○
Has an active imagination	○	○	○	○	○
Tends to be quiet	○	○	○	○	○
Is generally trusting	○	○	○	○	○
Tends to be lazy	○	○	○	○	○
Is emotionally stable, not easily upset	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is inventive	○	○	○	○	○
Has an assertive personality	○	○	○	○	○
Can be cold and aloof	○	○	○	○	○
Perseveres until the task is finished	○	○	○	○	○
Can be moody	○	○	○	○	○
Values artistic, aesthetic experiences	○	○	○	○	○
Is sometimes shy					

Below is a second set of statements about personality. For each item, please select the option that best indicates how much you agree with the following statements as they apply to you.

I am someone who...

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is talkative	○	○	○	○	○
Tends to find fault with others	○	○	○	○	○
Does a thorough job	○	○	○	○	○
Is depressed, blue	○	○	○	○	○
Is original, comes up with new ideas	○	○	○	○	○
Is reserved	○	○	○	○	○
Is helpful and unselfish with others	○	○	○	○	○
Can be somewhat careless	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is relaxed, handles stress well	○	○	○	○	○
Is curious about many different things	○	○	○	○	○
Is full of energy	○	○	○	○	○
Starts quarrels with others	○	○	○	○	○
Is a reliable worker	○	○	○	○	○
Can be tense	○	○	○	○	○
Is ingenious, a deep thinker	○	○	○	○	○
Generates a lot of enthusiasm	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Has a forgiving nature	○	○	○	○	○
Tends to be disorganized	○	○	○	○	○
Worries a lot	○	○	○	○	○
Has an active imagination	○	○	○	○	○
Tends to be quiet	○	○	○	○	○
Is generally trusting	○	○	○	○	○
Tends to be lazy	○	○	○	○	○
Is emotionally stable, not easily upset	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Is inventive	○	○	○	○	○
Has an assertive personality	○	○	○	○	○
Can be cold and aloof	○	○	○	○	○
Perseveres until the task is finished	○	○	○	○	○
Can be moody	○	○	○	○	○
Values artistic, aesthetic experiences	○	○	○	○	○
Is sometimes shy, inhibited	○	○	○	○	○
Is considerate and kind	○	○	○	○	○

Below is a third set of statements about personality. For each item, indicate to what extent you agree or disagree that the statement would have applied to you in a typical month *before* your ALS/MND diagnosis.

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I worried a lot of the time	○	○	○	○	○
I found it difficult to make a decision	○	○	○	○	○
I often felt jumpy	○	○	○	○	○
I found it hard to relax	○	○	○	○	○
I often could not enjoy things because of my worries	○	○	○	○	○
Little things bothered me a lot	○	○	○	○	○
I often felt like I have butterflies in my stomach	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I thought of myself as a worrier	○	○	○	○	○
I couldn’t help worrying about even trivial things	○	○	○	○	○
I often felt nervous	○	○	○	○	○
My own thoughts often made me anxious	○	○	○	○	○
I got an upset stomach due to my worrying	○	○	○	○	○
I thought of myself as a nervous person	○	○	○	○	○
I always anticipated the worst would happen	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I often felt shaky inside	○	○	○	○	○
I thought that my worries interfere with my life	○	○	○	○	○
My worries often overwhelmed me	○	○	○	○	○
I sometimes felt a great knot in my stomach	○	○	○	○	○
I missed out on things because I worry too much	○	○	○	○	○
I often felt upset	○	○	○	○	○

Below is a third set of statements about personality. For each item, indicate to what extent you agree or disagree that the statement applies to your experience over the past month.

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I worried a lot of the time	○	○	○	○	○
I found it difficult to make a decision	○	○	○	○	○
I often felt jumpy	○	○	○	○	○
I found it hard to relax	○	○	○	○	○
I often could not enjoy things because of my worries	○	○	○	○	○
Little things bothered me a lot	○	○	○	○	○
I often felt like I have butterflies in my stomach	○	○	○	○	○
	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I thought of myself as a worrier	○	○	○	○	○
I couldn't help worrying about even trivial things	○	○	○	○	○
I often felt nervous	○	○	○	○	○
My own thoughts often made me anxious	○	○	○	○	○
I got an upset stomach due to my worrying	○	○	○	○	○
I thought of myself as a nervous person	○	○	○	○	○
I always anticipated the worst would happen	○	○	○	○	○
	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I often felt shaky inside	○	○	○	○	○
I thought that my worries interfere with my life	○	○	○	○	○
My worries often overwhelmed me	○	○	○	○	○
I sometimes felt a great knot in my stomach	○	○	○	○	○
I missed out on things because I worry too much	○	○	○	○	○
I often felt upset	○	○	○	○	○

LIFE EVENTS

LIFE EVENTS

We would like to have some information about significant events during your life.

Please indicate which of the following events occurred by selected the age you were when it occurred. If it occurred more than once, select each age that it occurred (up to 5 ages).

The $200,000 mentioned in two questions is in United States dollars. Please convert this (approximately) into the currency you use to answer these questions.

	Age at First Occurrence	Age at Second Occurrence	Age at Third Occurrence	Age at Fourth Occurrence	Age at F Occurre
My spouse died					
I got a divorce					
I separated from my spouse					
I spent time in jail					
A close family member died (not a spouse)					
I had a major illness or injury					
I got married					
I was fired at work					
I had a marital reconciliation					
I retired					
A family member experienced a change in health (not myself)					
I became pregnant					
I experienced sexual problems					
My family gained a new member					
I experienced an upheaval in my business affairs					
I experienced a change in my financial state					
A close friend died					
I changed to a different occupation					
My spouse and I argued significantly more (or significantly less)					
I obtained a loan or mortgage over $200,000					
A loan or mortgage of mine was foreclosed					
My responsibilities at work significantly changed					
My son or daughter left home					
I had trouble with my in-laws					
I had an outstanding personal achievement					
My spouse began or stopped working					
I started or finished high school or college					
I had a significant change in my living conditions					
I had significant trouble with my boss					
My working hours or conditions changed significantly					
I changed residences					
I changed schools					
I changed recreational activities					

FAMILY

FAMILY

We would like to ask you some questions about your family background and medical history.

In which countries were your father and mother born?

	Country
Father's country of birth:	
Mother's country of birth:	

Please enter your parents' years of birth, years they died, and causes of death, as applicable. If they are still living, leave the year died and cause of death blank.

	Year	I don't know
Father's year of birth		
If your father has died, the year he died. Cause of death:		
Mother's year of birth		
If your mother has died, the year she died. Cause of death:		

Did your parents ever smoke cigarettes?

	Yes	No	I don't know
Mother			
Father			

What were your parents' main occupations during their lifetimes? If you do not know one or both of these answers, please answer "Not known" in the applicable boxes.

Father's main occupation	
Mother's main occupation	

We would like to get an indication of the size of your family. Please count the number of your relatives within the following groups:

1. First degree relatives: parents (always 2), brothers and sisters, and children.

Select number of first degree relatives:

2. Second degree relatives: uncles and aunts, nephews and nieces, grandparents (always 4), grandchildren, and half-brothers and half-sisters.

Select number of second degree relatives:

3. Third degree relatives: first cousins, great-grandparents (always 8), great-uncles (brothers of your grandparents) and great-aunts (sisters of your grandparents), grand-nephews and grand-nieces (children of your nephews and nieces) and great-grandchildren.

Select number of third degree relatives:

How many brothers and sisters (present and past) do you have? Please only include full siblings (that is, siblings with same mother and father as yourself).

Where do you come in the order of births of your brothers and/or sisters?

Do you have, or have you ever had, any biological children?

- Yes
- No

How many biological children do you have/have you had in total?

Please enter the number of relatives of yours with ALS/MND, in the following groups:

1. First degree relatives: your parents, brothers and sisters, and children.

Number of your *male* first degree relatives with ALS/MND

Number of your *female* first degree relatives with ALS/MND

2. Second degree relatives: your uncles and aunts, nephews and nieces, grandparents, grandchildren, and half-brothers and half-sisters.

Number of your *male* second degree relatives with ALS/MND

Number of your *female* second degree relatives with ALS/MND

3. Third degree relatives: your first cousins, great-grandparents, great-uncles (brothers of your grandparents) and great-aunts (sisters of your grandparents), grand-nephews and grand-nieces (children of your nephews and nieces) and great-grandchildren.

Number of your *male* third degree relatives with ALS/MND

Number of your *female* third degree relatives with ALS/MND

4. More distant relatives:

Number of your *male* more distant relatives with ALS/MND

Number of your *female* more distant relatives with ALS/MND

Have any of your relatives (either present or past) been diagnosed with the specific form of dementia, *frontotemporal* dementia?

- Yes
- No
- I don't know

Please enter the number of relatives of yours with frontotemporal dementia, in the following groups:

1. First degree relatives: your parents, brothers and sisters, and children.

Number of your first degree relatives with frontotemporal dementia

2. Second degree relatives: your uncles and aunts, nephews and nieces, grandparents, grandchildren, and half-brothers and half-sisters.

Number of your second degree relatives with frontotemporal dementia

3. Third degree relatives: your first cousins, great-grandparents, great-uncles (brothers of your grandparents) and great-aunts (sisters of your grandparents), grand-nephews and grand-nieces (children of your nephews and nieces) and great-grandchildren.

Number of your third degree relatives with frontotemporal dementia

4. More distant relatives:

Number of your more distant relatives with frontotemporal dementia

Have any of your relatives with ALS/MND or frontotemporal dementia had a positive gene test for a known ALS/MND gene?

- Yes
- No
- I don't know

How many of your relatives with ALS/MND or frontotemporal dementia have had a positive gene test for a known ALS/MND gene?

Have any of your relatives (either present or past) been diagnosed with ALS/MND?

- Yes
- No
- I don't know

Please enter the number of relatives of yours with ALS/MND, in the following groups:

1. First degree relatives: your parents, brothers and sisters, and children.

Number of your *male* first degree relatives with ALS/MND

Number of your *female* first degree relatives with ALS/MND

2. Second degree relatives: your uncles and aunts, nephews and nieces, grandparents, grandchildren, and half-brothers and half-sisters.

Number of your *male* second degree relatives with ALS/MND

Number of your *female* second degree relatives with ALS/MND

3. Third degree relatives: your first cousins, great-grandparents, great-uncles (brothers of your grandparents) and great-aunts (sisters of your grandparents), grand-nephews and grand-nieces (children of your nephews and nieces) and great-grandchildren.

Number of your *male* third degree relatives with ALS/MND

Number of your *female* third degree relatives with ALS/MND

4. More distant relatives:

Number of your *male* more distant relatives with ALS/MND

Number of your *female* more distant relatives with ALS/MND

Have any of your relatives (either present or past) been diagnosed with the specific form of dementia, *frontotemporal* dementia?

- Yes
- No
- I don't know

Please enter the number of relatives of yours with frontotemporal dementia, in the following groups:

1. First degree relatives: your parents, brothers and sisters, and children.

Number of your first degree relatives with frontotemporal dementia

2. Second degree relatives: your uncles and aunts, nephews and nieces, grandparents, grandchildren, and half-brothers and half-sisters.

Number of your second degree relatives with frontotemporal dementia

3. Third degree relatives: your first cousins, great-grandparents, great-uncles (brothers of your grandparents) and great-aunts (sisters of your grandparents), grand-nephews and grand-nieces (children of your nephews and nieces) and great-grandchildren.

Number of your third degree relatives with frontotemporal dementia

4. More distant relatives:

Number of your more distant relatives with frontotemporal dementia

Have any of your relatives with ALS/MND or frontotemporal dementia had a positive gene test for a known ALS/MND gene?

- Yes
- No
- I don't know

How many of your relatives with ALS/MND or frontotemporal dementia have had a positive gene test for a known ALS/MND gene?

Have any of your *first degree* relatives (parents, brothers and sisters, or children) suffered from any of the following neurological conditions (apart from ALS/MND)?

	Yes	No	Number of First Degree Relatives
Dementia (any type)	○	○	
Parkinson's Disease	○	○	
Multiple Sclerosis	○	○	
Other brain or spinal cord disorder (please specify):			
	○	○	
Other brain or spinal cord disorder (please specify):			
	○	○	
Other brain or spinal cord disorder (please specify):			
	○	○	

Have any of your *first degree* family (parents, brothers and sisters, children) suffered any other major diseases (not involving the brain or spinal cord)? Please list the name the disease in the left column and select the applicable family member in the right column.

	Name of Disease	Family Member
Disease 1		
Disease 2		
Disease 3		
Disease 4		
Disease 5		
Disease 6		
Disease 7		
Disease 8		
Disease 9		
Disease 10		

ALS/MND CLINICAL INFORMATION

ALS/MND CLINICAL INFORMATION

Now we are going to ask you some questions about your ALS/MND.

In which month and what year did you get your first *symptom* of ALS/MND?

	Month	Year
Date of first symptom		

What was the first symptom of ALS/MND that you noticed?

- Right upper limb muscle weakness
- Left upper limb muscle weakness
- Right lower limb muscle weakness
- Left lower limb muscle weakness
- Muscle twitches (fasciculations)
- Slurred speech
- Difficultly swallowing
- Shortness of breath
- Spasticity (increased muscle tone)
- Other (please specify):

In which month and what year were you *diagnosed* as having ALS/MND?

	Month	Year
Date of diagnosis		

We would like to know what type of ALS/MND you have.

If you do not know the type of ALS/MND you have (listed below) please contact your neurologist or family doctor/general practitioner to ask for this information.

You can mention the types of ALS/MND listed below (with their patterns) when you contact your neurologist or family doctor and ask them to pick which one you have.

Note that a diagnosis of "ALS" or "MND" by itself may not give information about the type of the disease you have, because confusingly these two terms are often used to denote all the types of ALS/MND together.

What type of ALS/MND have you been diagnosed as having?

- Amyotrophic lateral sclerosis (ALS, also called "classic" ALS)
  Pattern: Mixed upper and lower motor neuron signs in limbs and bulbar region
- Progressive muscular atrophy
  Pattern: Pure lower motor neuron syndrome
- Primary lateral sclerosis
  Pattern: Pure upper motor neuron syndrome
- Progressive bulbar palsy
  Pattern: Isolated upper or lower motor neuron signs, or both, only in bulbar muscles (which are involved, for example, in speech and swallowing)
- ALS/MND and frontotemporal dementia
  Pattern: A combination of any type of ALS/MND and frontotemporal dementia
- Flail arm syndrome
  Pattern: Symmetrical, proximal, largely lower motor neuron weakness of upper limbs
- Flail leg syndrome
  Pattern: Symmetrical, distal, largely lower motor neuron weakness of lower limbs
- Other (please specify):

- I do not know what type of ALS/MND I have been diagnosed with.

Have you had a positive gene test for a known ALS/MND gene?

- Yes
- No
- I don't know

Has your personality changed since you were diagnosed with ALS/MND?

- Yes
- No

Please describe your personality 10 years before your ALS/MND diagnosis, and now.

10 years before diagnosis	
Now	

Please compare your memory now, to what it was before you got your first symptom of ALS/MND. We understand this might be difficult, but we are interested in your opinion.

- Better
- Much Better
- About the Same
- Worse
- Much Worse
- I am unable to make a comparison

Now please ask somebody who knows you well (such as partner, relative or close friend) to compare your memory now, to what it was before you got your first symptom of ALS/MND.

- Better
- Much Better
- About the Same
- Worse
- Much Worse
- They are unable to make a comparison

We would like to get a measure of your current functional status.

Please select one of the options from each of the following 12 ALS/MND Functional Rating Scale items.

1. Speech

- Normal speech processes
- Detectable speech disturbance
- Intelligible with repeating
- Speech combined with non-vocal communication
- Loss of useful speech

2. Salivation

- Normal
- Slight but definite excess of saliva in mouth; may have night-time drooling
- Moderately excessive saliva; may have minimal drooling
- Marked excess of saliva with some drooling
- Marked drooling; requires constant tissue or handkerchief

3. Swallowing

- Normal eating habits
- Early eating problems - occasional choking
- Dietary consistency changes
- Needs supplemental tube feeding
- Nothing by mouth (exclusively tube or intravenous feeding)

4. Handwriting

- Normal
- Slow or sloppy; all words are legible
- Not all words are legible
- Able to grip pen but unable to write
- Unable to grip pen

5. Cutting food

- Normal
- Somewhat slow and clumsy, but no help needed
- Can cut most foods, although clumsy and slow; some help needed
- Food must be cut by someone, but can still feed slowly
- Needs to be fed

6. Dressing and hygiene

- Normal
- Independent and complete self-care with effort or decreased efficiency
- Intermittent assistance or substitute methods
- Needs attendant for self-care
- Total dependence

7. Turning in bed

- Normal
- Somewhat slow and clumsy, but no help needed
- Can turn alone or adjust sheets, but with great difficulty
- Can initiate, but not turn or adjust sheets alone
- Helpless

8. Walking

- Normal
- Early walking difficulties
- Walks with assistance
- Non-walking functional movement only
- No purposeful leg movement

9. Climbing stairs

- Normal
- Slow
- Mild unsteadiness or fatigue
- Needs assistance
- Cannot do

10. Shortness of breath

- None
- Occurs when walking
- Occurs with one or more of the following: eating, bathing, dressing
- Occurs at rest, difficulty breathing when either sitting or lying
- Significant difficulty, considering using mechanical respiratory support

11. Shortness of breath on lying flat

- None
- Some difficulty sleeping at night due to shortness of breath. Does not routinely use more than two pillows
- Needs extra pillow in order to sleep (more than two)
- Can only sleep sitting up
- Unable to sleep

12. Respiratory insufficiency

- None
- Intermittent use of positive air pressure device
- Continuous use of positive air pressure device
- Continuous use of positive air pressure device during the night and day
- Invasive mechanical ventilation by intubation or tracheostomy

What factor or factors do *you* think could have played a part in your getting ALS/MND? You may list up to three in the boxes below.

Factor 1

Factor 2

Factor 3

If answering this questionnaire has raised any issues of concern for you, we suggest that you contact your local ALS/MND Support Group or Association.

SUBMISSION OF QUESTIONNAIRE

SUBMISSION OF QUESTIONNAIRE

This is the last page of the questionnaire.

If you want to continue entering answers or change answers in the questionnaire, use the Table of Contents icon (top left) or the Back button (<<) to navigate through it.

If you have finished the questionnaire, click the "Submit" button below to record your response. You will not be able to access your questionnaire answers again once you have done this, so please ensure your information is complete and accurate before submitting.

If you have any questions, comments or suggestions on this questionnaire, please email als.quest@sydney.edu.au

Thank you very much for your help with this research.

APPENDIX B

Lessons learned from translating a global web-based questionnaire into different languages

LESSONS LEARNED FROM TRANSLATING A GLOBAL WEB-BASED QUESTIONNAIRE INTO DIFFERENT LANGUAGES

1. introduction

An online questionnaire for research into potential risk factors for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND)[1] using the Qualtrics Research Suite software has been developed.[2] One available feature of this software is the ability to translate the questionnaire into many languages via Google Translate. One of the aims in developing the questionnaire was to make it accessible to as many of the people in the world as possible, because there are many places where potential risk factors for ALS/MND have not been investigated. In addition, having a questionnaire that is available virtually world-wide will also allow for comparisons of risk factors among different countries.

This article summarises the process that was used to undertake the task of translating the questionnaire into an additional 27 languages from its original English version, including the languages spoken in countries that are members of the International Alliance of ALS/MND Associations. Typical results in the literature have focused on the translation of a questionnaire into a single other language (or group of languages from a particular region) (see, for example: [3-16]); this is the first summary that documents the translation of an online questionnaire for global distribution.

2. Methods

2.1. Choosing target languages for translations

2.1.1. Languages spoken in Australia

The questionnaire is based in Australia. Therefore, it was desirable to make the questionnaire accessible to as many Australians as possible. The languages (other than English) most commonly spoken in Australian homes, based on the 2011 Census [17], were identified and included on the list of desired translations. These languages are: Arabic, simplified Chinese (Mandarin and Cantonese are both on the list; however,

written Chinese is largely the same for these dialects), Greek, Hindi, Italian, Spanish, and Vietnamese. Tagalog is also on this list; however, because English is an official language in the Philippines and because Tagalog is not available on Qualtrics translation tool, it was decided not to include it.

2.1.2. Languages spoken in countries that are members of the International Alliance of ALS/MND Associations

A list of members of the International Alliance of ALS/MND Associations was compiled from the Association's directory [18] and the official/commonly-spoken languages in these countries were identified. The languages identified from this task were: Afrikaans, Croatian, Danish, Dutch, Finnish, French, German, Icelandic, Japanese, Korean, Latvian, Mongolian, Persian, Polish, Portuguese, Brazilian Portuguese, Romanian, Serbian, Slovenian, Swedish, and Turkish. (Some Alliance languages overlapped with languages identified for Australia; these are not listed here.)

2.1.3. Other

In considering the list of languages identified in the first two steps and its geographical reach, some gaps were observed. To remedy this, several other languages were added, with the intent to reach as large a population as possible, including in particular those people proximal to Australia. The languages identified in this category were: Czech, Hebrew, Hungarian, Indonesian, Malaysian, Norwegian, Russian, and Thai.

2.2. Implementing the initial translations

Using Qualtrics' Translate Survey tool, a questionnaire can be translated into a selected group of languages via Google Translate [19]. The precise list of available languages for the translations is determined internally by Qualtrics (but can by bypassed by using the technique described below).

The initial translations of the questionnaire were conducted once the English version of the questionnaire was final. This was to avoid making edits to the individual translated versions.

In the first translation attempt, the desired languages were added directly onto the existing English version, so that one version of the questionnaire contained all available translations. The initial translations were performed by going question by question and translating each question into the desired languages, one at a time. The translation process itself is simple, consisting of accessing the "Translate Survey" option in Qualtrics for the questionnaire, selecting the language of the translation, and clicking a button. Qualtrics then sends the question and answer text from their server to the Google Translate server, and the translated version is then sent back to replace the English text in the translated version. The translated text is automatically shown next to the original English version for a side-by-side comparison. The text can then be edited if necessary to achieve a more accurate translation. While there is some manual aspect to this process, it is largely a commitment of time rather than effort.

One limitation of the Qualtrics Translate Survey tool is that there is no capability to redo a translation, so if changes were made the original English version, the text cannot be re-translated to reflect these edits. Rather, the translation must be edited manually.

2.3. Technical issues and resolution

After the original English questionnaire had been translated into approximately 20 languages, an internal server error developed. The result of this error was that the questionnaire in its entirety was no longer able to be viewed or edited on the Qualtrics website. After some investigation by Qualtrics, it was discovered that the database file that had been created by the questionnaire and its many translations had exceeded a size that could be handled by the Qualtrics Servers. Unfortunately, the current version of the questionnaire was not able to be restored; fortunately, however, it was still functioning online so that the data collection process was not impacted.

Qualtrics was able to recover the vast majority of the original database file, including the translations that had been performed to date, with the exception of one question (which had to be re-translated). To avoid a recurrence of the database issue, it was decided to create 36 separate database files (i.e., 36 separate questionnaires), one for each language. This was viewed as a prudent risk reduction measure to minimise the

loss that would occur should any particular questionnaire and its translation encounter a technical issue. Once all the issues were resolved, the English version was relaunched as an English-only version and the malfunctioning questionnaire was deactivated. Because there was no longer the capability to offer all the translations in a single questionnaire, a new survey was created to overlay onto all the other questionnaires, which consisted of one question asking which language in which the respondent would like to complete the main questionnaire. Based on this selection, the website then directs the respondent to the web address associated with the questionnaire for that specific translation.

One advantage of this approach is that the data for a specific language can be downloaded for review in a specific batch, such that the data for the different languages is not comingled. This might also simplify country-by-country comparisons to some extent. One disadvantage of this approach is that numerical and scale data, which are identical in all the translated versions, would need to be compiled for all the translations, whereas in the initial approach, these data would have been contained in one database and available for download as one unit.

2.4. Languages not available using Qualtrics Translate Survey tool

Two languages identified as desired translations were not available in Qualtrics built-in Translate Survey tool: Afrikaans and Icelandic. Qualtrics does, however, allow for adding languages that are not on their default list, and the corresponding translation must be completed outside of Qualtrics. As Afrikaans and Icelandic are included in the list of languages on Google Translate, these translations were performed as follows. First, the English file of the original questionnaire was downloaded from Qualtrics. Then, the file was translated using the Google Translate feature that allows one to translate a document. Finally, the translated file was uploaded back into Qualtrics to become the new version of the translated questionnaire.

Smaller translation tasks (e.g., translating the first and second pages of the questionnaire) were performed by copying the text from Qualtrics into Google Translate and copying back the translated version.

2.5. Validating of the Google translations

For optimal intelligibility and accuracy, the Google-translated versions need to be checked by fluent speakers of each language. As one example, in a question about skin color, the word "fair" in most languages is translated as "reasonable" rather than the intended meaning of "light in colour". To disseminate a Google translated version of a questionnaire without checking it would be possible, but not advisable.

As the first task, the simplified Chinese translation was checked and validated, which is one of the languages where Google Translate appeared to give the greatest number of ambiguities. A colleague in the organisation conducted this check so that the researchers could work closely with her to see how the checking process functions and identify any potential technical and/or textual issues.

Although it had been endeavored to avoid questions that could be related to particular cultures or backgrounds, one issue arose during the check of the Chinese language version for a question in the ALS Functional Rating Scale. The question asks about the ALS/MND patient's ability to cut food, which is not generally applicable to Chinese who eat with chopsticks. To address this, the text of the question was adapted to state: "Cutting food (Western style)." Similar issues have not been identified to date in reviews of other languages.

The Google-translated languages that have been checked for accuracy are listed in the list of available translations of the questionnaire on the ALS Quest website (alsquest.org).

2.5.1. Finding volunteer translators

There are a few potential options for undertaking the checking and validating of the questionnaire translations. The choice might depend on where one falls on the cost-speed-quality continuum. For speed and quality, one option would be to use Qualtrics' "Paid Translations" service, where the questionnaire will be translated and checked by

two native speakers of a language, or otherwise hire a professional translator to check the translation. However, this would likely be the option with the highest cost.

As this study was located in a university setting with a large number of international students, it was decided to ask for volunteers from among the student population to check and validate the Google translated versions of our questionnaire. Dr. Pamphlett gives occasional lectures to medical and dental students, so he was able to ask these students directly about being potential volunteers. In addition, it was felt that students with these backgrounds might be more interested in assisting with an epidemiological study of a rare disease. Email recruitment was also conducted of medical school classes and students at another medical campus associated with the University of Sydney.

This approach of using volunteer translation checkers has the advantage of being essentially free. In general, it has been found that the quality of the work by our volunteers is very good. The most significant disadvantage of this approach is a lack of speed, as students tend to be quite busy and therefore can take weeks to months to complete the work. This has not been too great of a concern relative to this project, however, as the timeframe for collecting responses is anticipated to be at least five to ten years, thus rendering any delays largely immaterial.

2.5.2. Working with volunteer translators

The primary technical challenge to having the volunteers check the translations was providing them with access to the online questionnaire translation. One option would have been to provide them with access to the project's Qualtrics account; however, this would present certain unacceptable security risks, as then they would have access to all of our questionnaires and data.

The method for obviating these risks was to create separate user accounts for each translator. We were able to do this by adding an "Administration" feature to the Qualtrics account, which gave the capability to create additional user accounts under one master account. The administrator also has the ability to constrain the capabilities of the user accounts. In this case, the translation users are primarily limited to using the

translate survey feature. This minimises the risk that they might alter the questionnaire in some other way.

After creating the account, the version of the questionnaire that has been translated into the language that the volunteer is checking is "shared" with their account using Qualtrics "Collaborate" feature. Each of the accounts therefore has access to only one copy of the questionnaire (in one particular language) and does not have access to any of the project data.

To help the translators get started, a "user's manual" was developed to provide a summary of how the process of checking the translation works and to disseminate "tips" learned from prior translators in the course of conducting their translation checks.

In addition to checking the translation via Qualtrics website, other options for the translators include providing them with a csv file (that can be opened in Microsoft Excel) or an xml file of the translation, which they can then check and edit offline. Qualtrics allows a user to download a questionnaire in csv or xml, and then upload an edited file to replace the existing version of the questionnaire. Although most translators used the web-based checking option, the xml option was preferred by some and presented no particular technical challenges, as long as the checker did not alter any of the xml tags in the file.

3. Results and Discussion

3.1. Deploying the translations

In contrast to the main body of the questionnaire, which has individual survey files for each translation, the first and second web pages of the questionnaire are two single and separate pages that can be displayed in any of the available translations. In fact, each of these pages is set up as a "survey" on Qualtrics – the first page consists solely of a single text box survey "question," and the second page has only one question, which asks the respondents to select their preferred language for the main questionnaire.

After respondents have selected their preferred language from the list of available translations, they then enter the main body of the questionnaire. Participants can select

from any of the available languages, regardless of the language in which the questionnaire is displayed. Once one language is selected, the main body of the questionnaire is available to display only in the selected language and in English.

Of note is that the Qualtrics site automatically displays its pages in a particular language if one of the available translated languages is selected as default on a respondent's Internet browser. For example, a respondent who has their browser language set to Français will automatically see a French translation of the first and second pages of the questionnaire. Assuming that they then select Français as their desired language, the main body of the questionnaire will also display in French (without the respondent taking any specific action on their part).

For answers requiring text entry, respondents are able enter answers in their own language. Since only a few questions are answered by entering text, translations back into English will not be onerous.

3.2. Questionnaire dissemination

Once the questionnaire translations have been checked, the international ALS/MND community was made aware of their availability. To accomplish this, emailed letters were sent to the appropriate ALS/MND associations when the language of their country became available. ALS Quest also has a Facebook page [20] and a Twitter account [21], where updates are posted to announce when a language are added to the questionnaire. The International Alliance of ALS/MND Associations has also assisted this project by disseminating emails to their distribution list regarding ALS Quest and notifying their members of its availability in many languages.

3.3. Results to date

The greatest numbers of respondents by language are English, Spanish, Portuguese/Brazilian Portuguese, Russian, French, Dutch, Danish, German, Chinese, Romanian, Afrikaans, Korean, Finnish, Italian, Arabic, Persian, Indonesian, Serbian, Polish, Vietnamese, Czech, Japanese, Hebrew, and Swedish.

4. Acknowledgements

I would like to acknowledge the many volunteers who have assisted us with the translations of ALS Quest. Their contribution has been instrumental in making the questionnaire accessible to the greatest possible number of people.

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APPENDIX C

ALS Quest: Designing and implementing an international web-based questionnaire to look for risk factors for ALS/Motor Neuron Disease

(platform presentation at Alliance of International ALS/MND Associations, 2017)

ALS QUEST

Designing and implementing an international web-based questionnaire to look for risk factors for ALS/Motor Neuron Disease

SYDNEY MEDICAL SCHOOL

JANE PARKIN KULLMANN
NEUROPATHOLOGY | BRAIN AND MIND CENTRE

THE UNIVERSITY OF SYDNEY

ALS Quest Risk Factor Questionnaire

THE UNIVERSITY OF SYDNEY

ALS Quest

A QUESTIONNAIRE FOR RESEARCH INTO AMYOTROPHIC LATERAL SCLEROSIS AND MOTOR NEURON DISEASE

Welcome to ALS Quest.

ALS Quest is a research questionnaire developed by Doctor Roger Pamphlett at the University of Sydney to search for causes of amyotrophic lateral sclerosis.

We hope this project will find methods to prevent and to treat amyotrophic lateral sclerosis.

People with amyotrophic lateral sclerosis are invited to complete the questionnaire.

If you do not have amyotrophic lateral sclerosis, you are also invited to complete the questionnaire.

You will not be asked for any identifiable information.

Please select your language below and then click on the next button (>>) to begin or continue the questionnaire.

Our first publication – August 2015

JMIR RESEARCH PROTOCOLS Parkin Kullmann et al

Original Paper

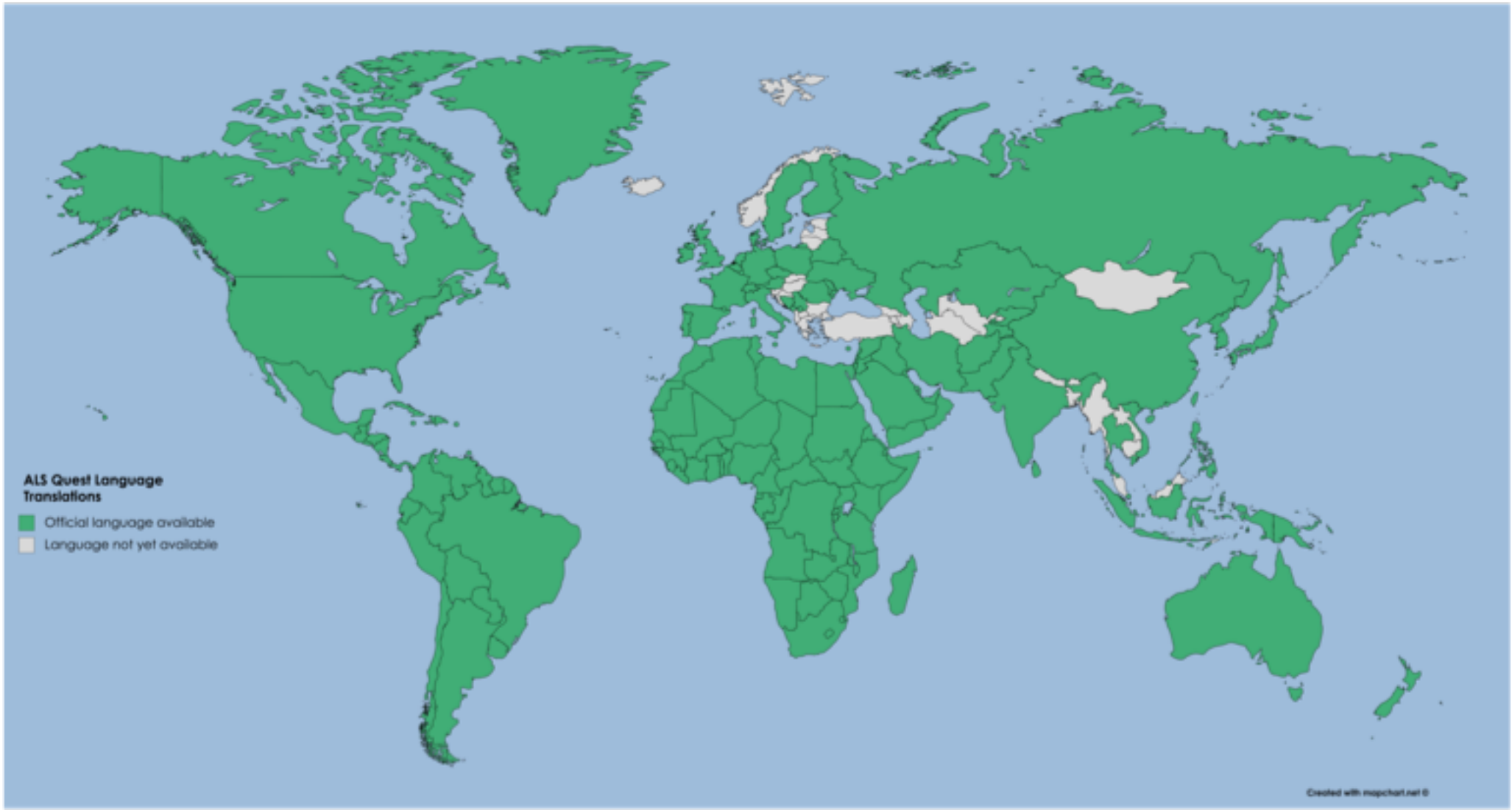
Designing an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study

Jane Alana Parkin Kullmann, AB, MS; Susan Hayes, PhD; Min-Xia Wang, MD; Roger Pamphlett, MBChB, MD

The University of Sydney, Camperdown NSW, Australia

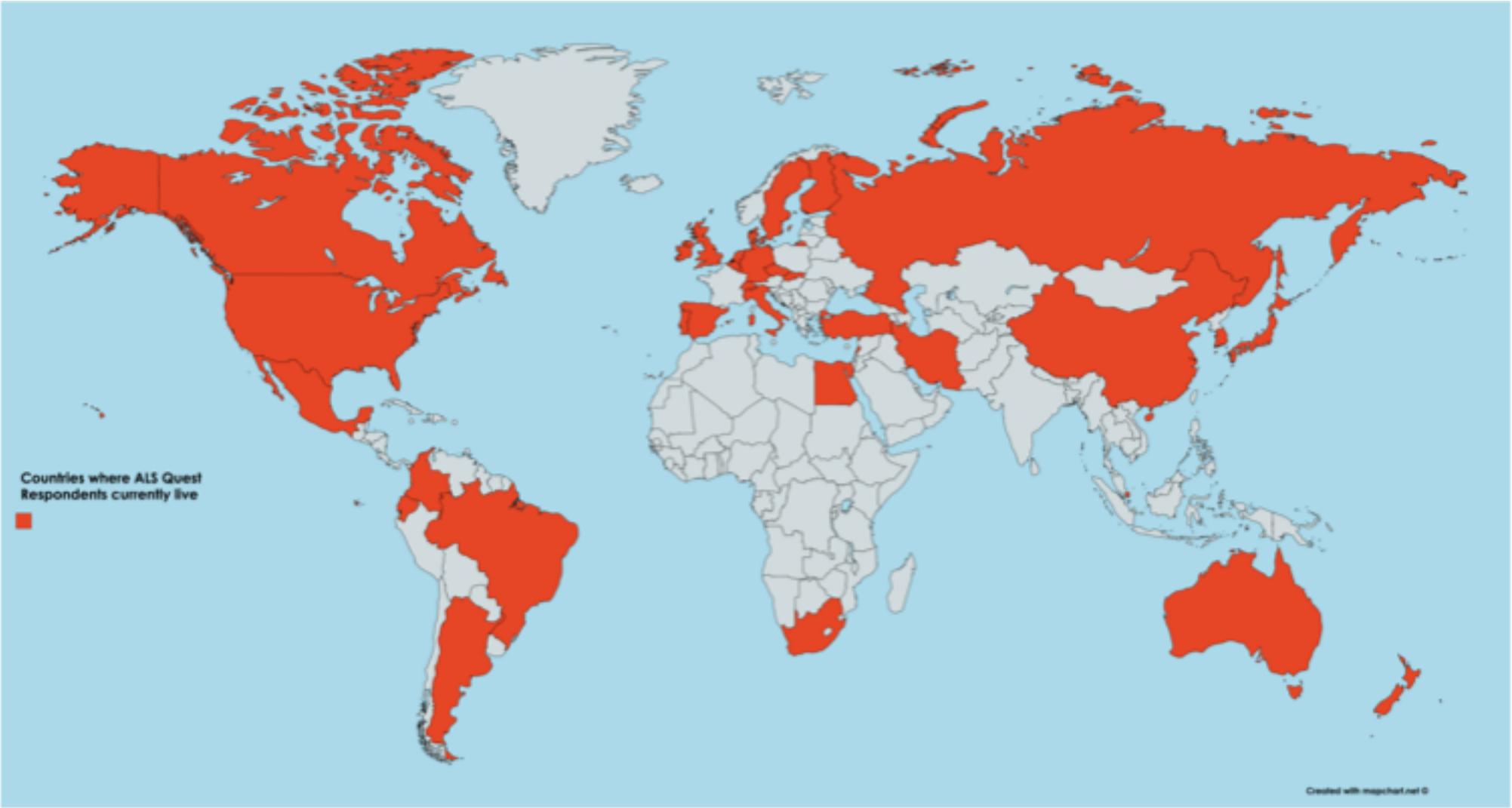
Full text freely available at:
http://www.researchprotocols.org/2015/3/e96/

Translations



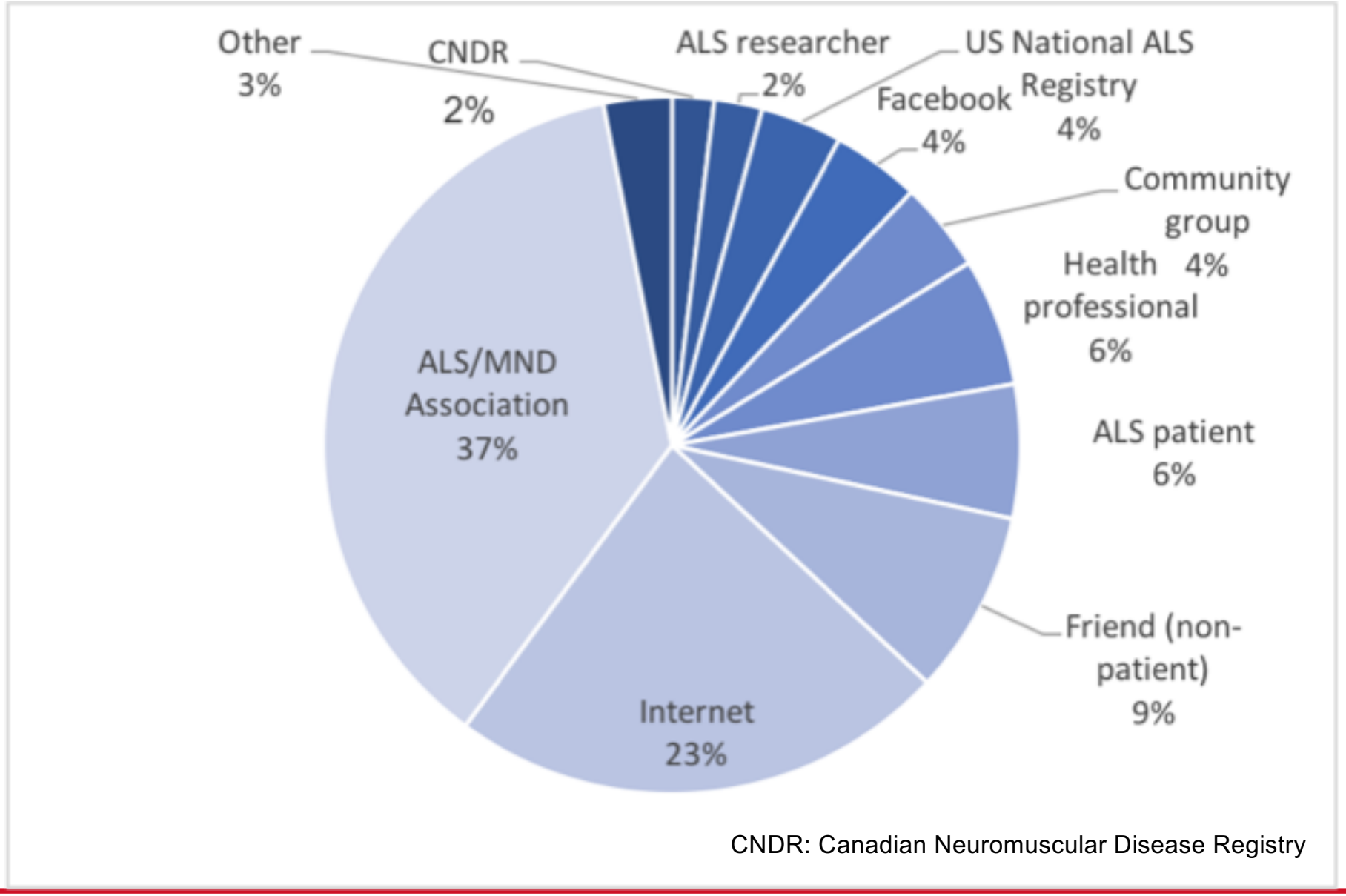
Countries in **green** are those in which ALS Quest can be completed in at least one official language

Respondent Demographics



Countries in **red** are those in which residents have completed ALS Quest surveys

Sources of information about ALS Quest as reported by respondents



CNDR: Canadian Neuromuscular Disease Registry

Advantages

- Larger numbers of respondents possible at lower cost than other methods (e.g., paper, telephone)
- Study can be accessed worldwide
- Provides the ability to translate questionnaire into many languages
- Questionnaire responses are captured automatically in a secure online database

Limitations

- Cannot classify cases using El Escorial criteria nor independently confirm an ALS diagnosis; however, another study found 94 percent of respondents to an online questionnaire were accurate about their ALS diagnosis
- Unable to obtain biological samples from patients (e.g., DNA samples)

Results from ALS Quest

First results paper – August 2017

BMJ Open

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Article Text

Article info

Citation Tools

Neurology

Research

Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case–control study

Jane Alana Parkin Kullmann, Roger Pamphlett

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Full text freely available at:
http://bmjopen.bmj.com/content/7/8/e016924

What's next for ALS Quest

- Additional papers summarising the results of the study are forthcoming.

- **We are still collecting responses. T**he final aim is to have 5,000 people with ALS/MND and 5,000 controls complete the questionnaire.

  To date we have almost 1,100 completed responses, 430 ALS patients and 660 controls.

- The questionnaire is available to all at www.alsquest.org

- Find us on Facebook at: www.facebook.com/ALSQuestSydneyUni

- Follow us on Twitter: @ALS_Quest

APPENDIX D

ALS Quest: An international online questionnaire to find risk factors for ALS

(poster presentation at the International MND Symposium, 2017)

ALS Quest: an international online questionnaire to find risk factors for ALS

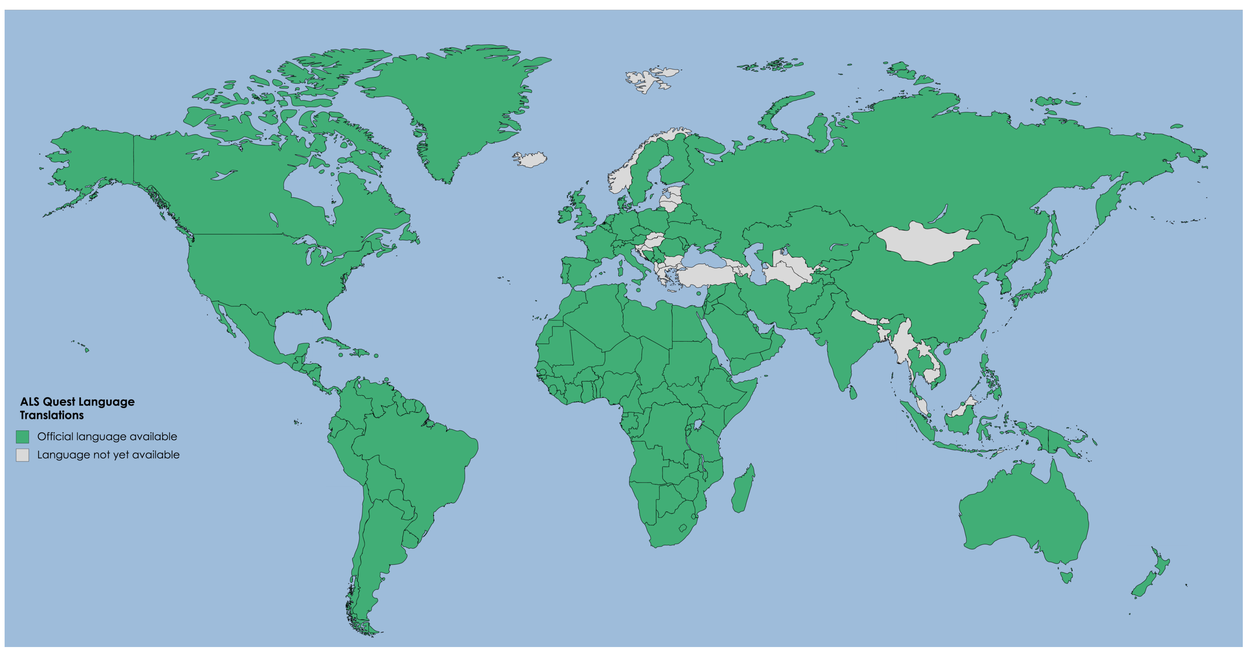
Jane Parkin Kullmann and Roger Pamphlett

An international survey to look for robust ALS risk factors

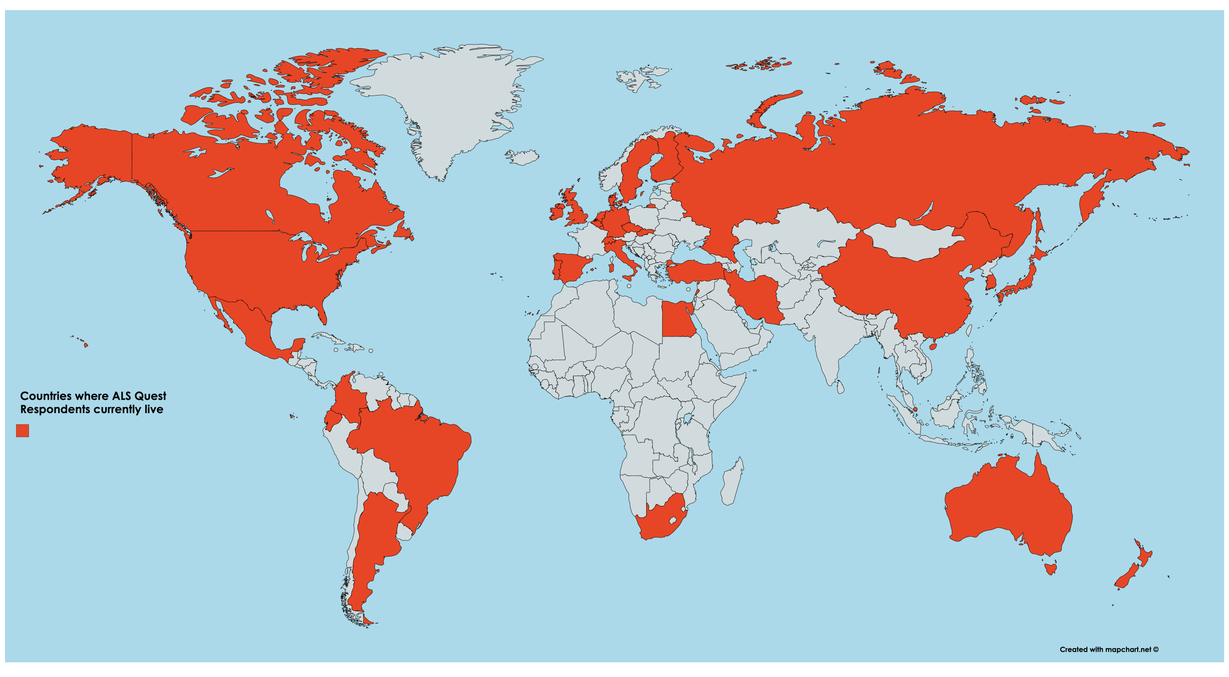
- To compare ALS risk factors between different countries, an online questionnaire available in multiple languages is needed
- ALS Quest was launched online in 2015 and to date more than 1,000 responses have been received
- The questionnaire will be active for 10 years, with the aim of collecting 5,000 ALS and 5,000 control responses

Methods

- Questions include details of occupations, chemical and pesticide exposures, physical activity, family sizes, medical histories, personality types, and life stressors
- The questionnaire (which has been translated into 27 languages) takes about 90 minutes to complete, and can be conducted in multiple sessions on computers, tablets and smart phones
- Recruitment is via several methods, including ALS/MND Associations, ALS Registries, the Internet, and social media

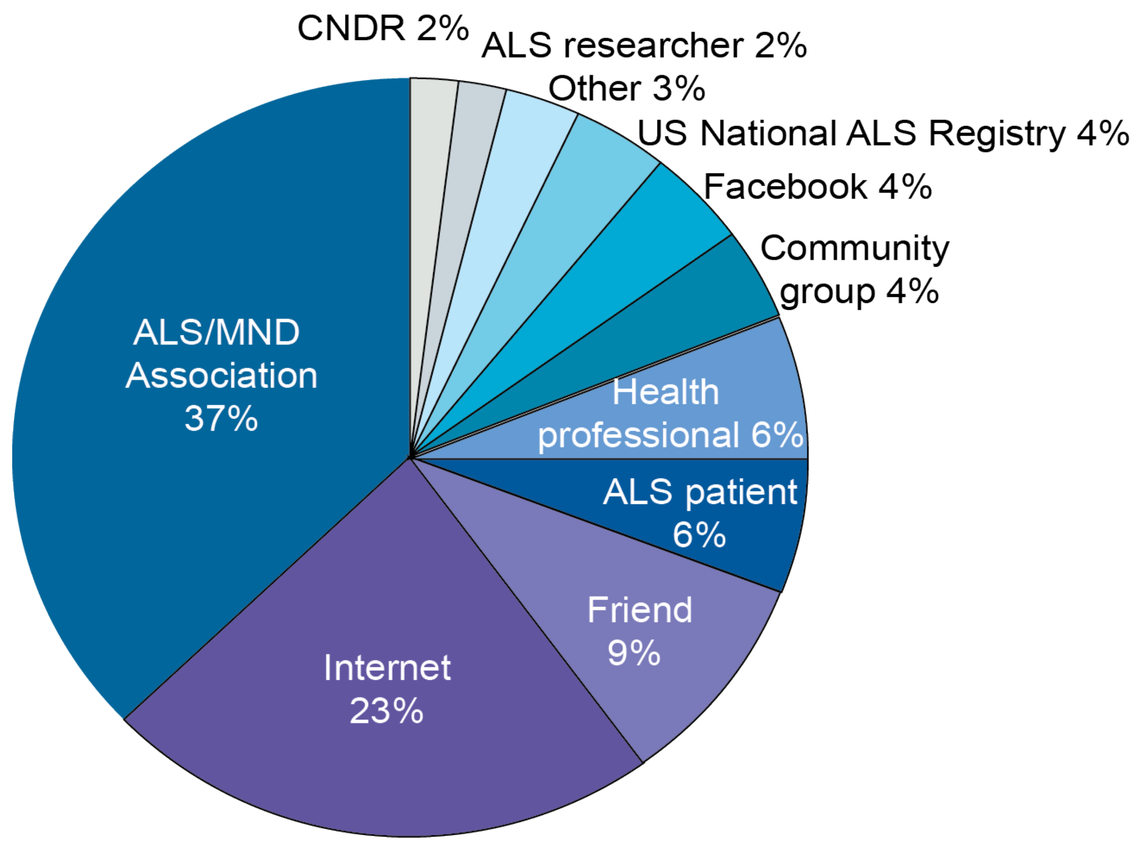


Countries in **green** are those in which ALS Quest can be completed in at least one official language



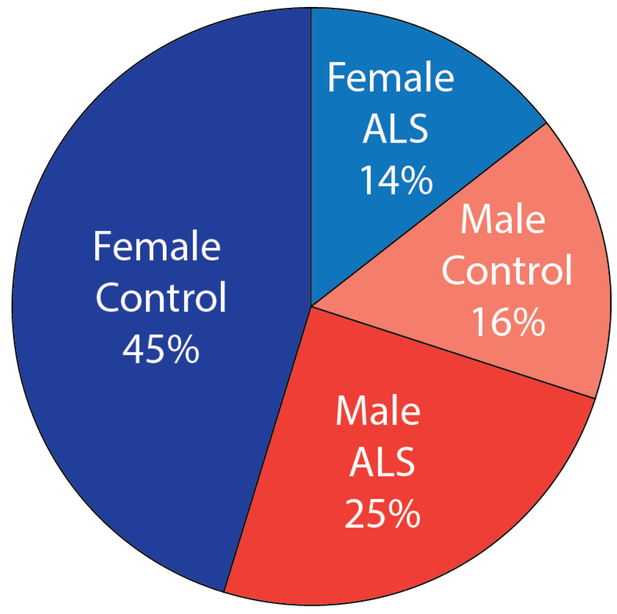
Countries in **red** are those in which residents have completed ALS Quest surveys

Respondents reported that they found out about ALS Quest from:



CNDR: Canadian Neuromuscular Disease Registry

Respondents to date



Progress

- Some risk factors are already being analysed with the current responses
- International comparisons will be feasible once more responses are received
- The aim is to make the data *open source,* once sufficient numbers of responses from different countries are received, so that researchers from around the world will have access to the data

ALS Quest would not be possible without:

- ALS/MND Associations and ALS Registries in many countries who have encouraged people to complete the questionnaire
- The generous donation of survey questions from a number of ALS researchers
- Our volunteer translators

Parkin Kullmann JA, Hayes S, Wang MX, Pamphlett R. Designing an Internationally Accessible Web-Based Questionnaire to Discover Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study. *JMIR Res Protoc*. 2015;4:e96.

APPENDIX E

ALS Quest: An international online questionnaire to find risk factors for ALS

(poster presentation at the MND Australia meeting, 2015)

ALS QUEST: AN INTERNATIONALLY-ACCESSIBLE WEB-BASED RISK FACTOR QUESTIONNAIRE FOR MOTOR NEURON DISEASE

THE UNIVERSITY OF SYDNEY

Jane Parkin Kullmann and Roger Pamphlett

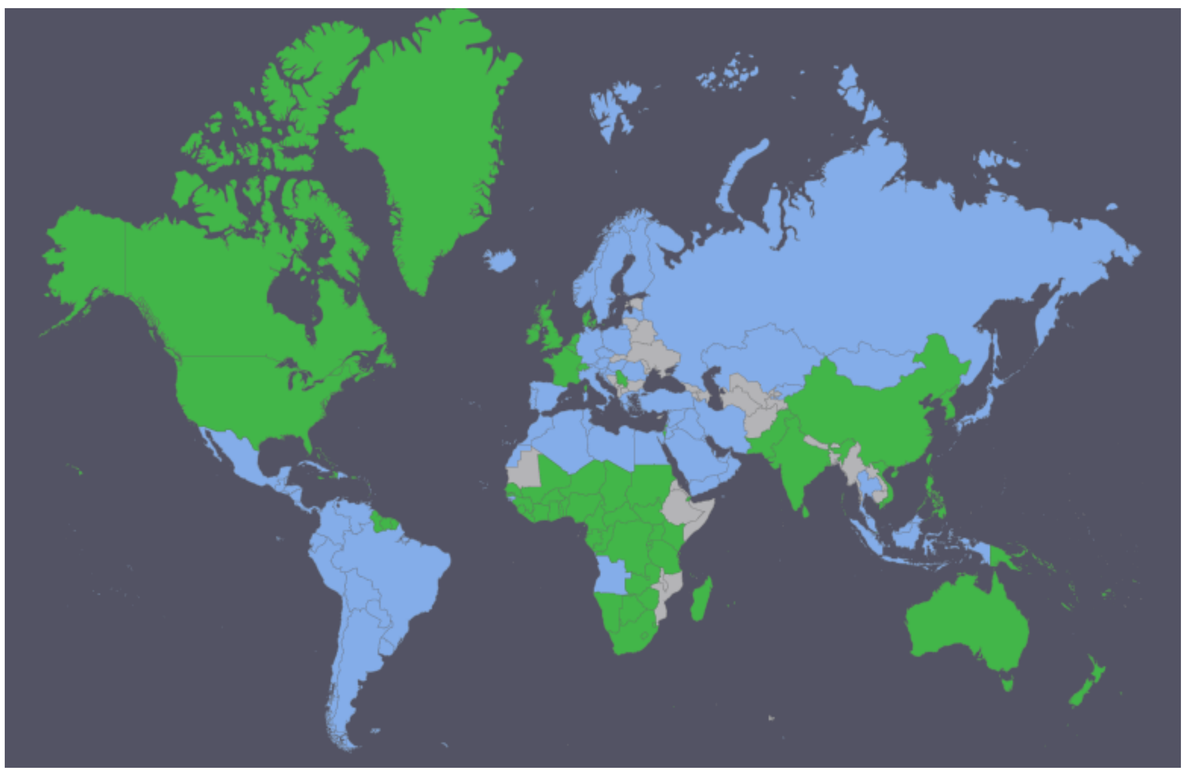
INTRODUCTION

Epidemiological studies using paper-based questionnaires have failed to find widely accepted risk factors for motor neuron disease (MND).

Web-based questionnaires have many advantages over paper-based ones, but few online epidemiological studies into neurodegenerative diseases have been undertaken.

METHODS

- We have developed a web-based questionnaire to identify environmental and lifestyle risk factors for MND
- Questions were included from the Australian MND DNA Bank questionnaire, literature searches, and validated MND questionnaires supplied by international investigators
- Risk factors that have not yet been extensively examined include prenatal exposure to testosterone (indicated by finger ratios), stress, personality type, and dental history
- The questionnaire is being translated into a number of languages to ensure international access and to find robust risk factors, i.e., those present in more than one country
- For more details please see our online publication, *Designing an internationally accessible Web-based questionnaire to discover risk factors for amyotrophic lateral sclerosis: a case-control study*. JMIR Res Protoc 2015



Available in official language of country: Currently By early 2016

PRELIMINARY RESULTS

After the questionnaire had been online for 4 months it had 379 respondents, compared to only 46 respondents for the same time period using a paper-based questionnaire.

Preliminary results are in line with previously-identified risk factors for MND, such as prenatal exposure to testosterone (as indicated by index to ring finger ratios) and exposure to certain chemicals.

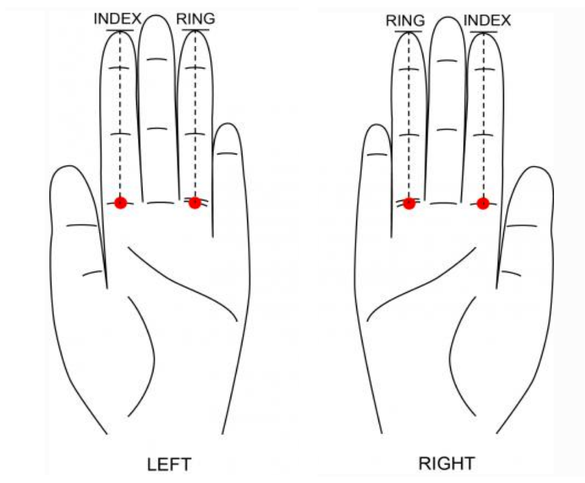
PRELIMINARY FINDINGS FOR INDEX:RING FINGER RATIOS

- Men with MND have *smaller* index:ring finger ratios
- Women with MND have *larger* index:ring finger ratios

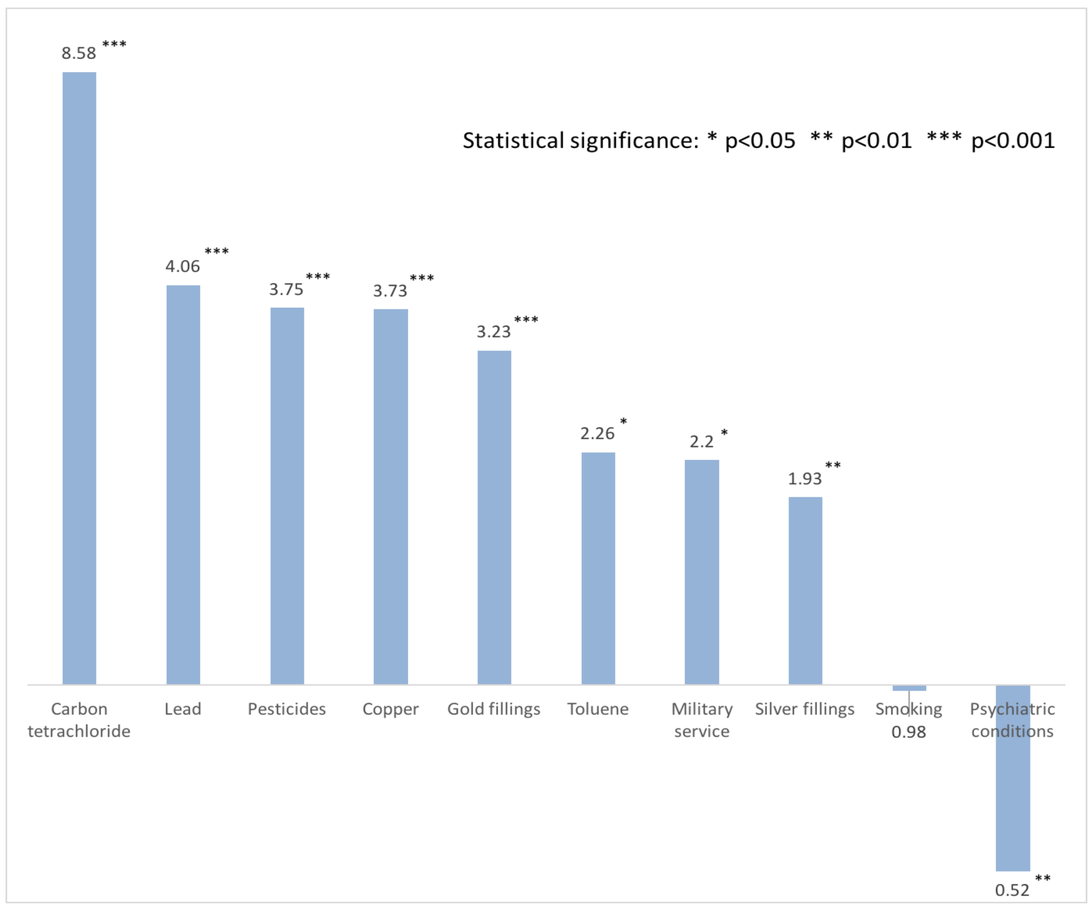
This suggests a link between finger ratio differences, MND, and prenatal exposure to hormones.

This diagram from the questionnaire shows how to measure fingers:

How do *your* finger ratios compare?



ODDS RATIOS FOR SELECTED ENVIRONMENTAL EXPOSURES



Bars above the baseline indicate factors that people with MND are more likely to have been exposed to, and those below the baseline less likely. Note that people with MND have smoked at the same rate as those without MND.

For all exposures, greater numbers of respondents are needed to determine *biological* significance.

CONCLUSIONS

- Early results from this questionnaire validate a number of risk factors for MND and suggest some new ones
- The questionnaire has the potential to identify more MND risk factors, and allow comparisons among a large number of countries to see how reproducible the factors are

APPENDIX F

Does the ratio of the index to the ring finger length (2D:4D) differ in ALS?

(poster presentation at the International MND Symposium, 2017)

DOES THE RATIO OF THE INDEX TO THE RING FINGER LENGTH (2D:4D) DIFFER IN ALS?

Jane Parkin Kullmann and Roger Pamphlett

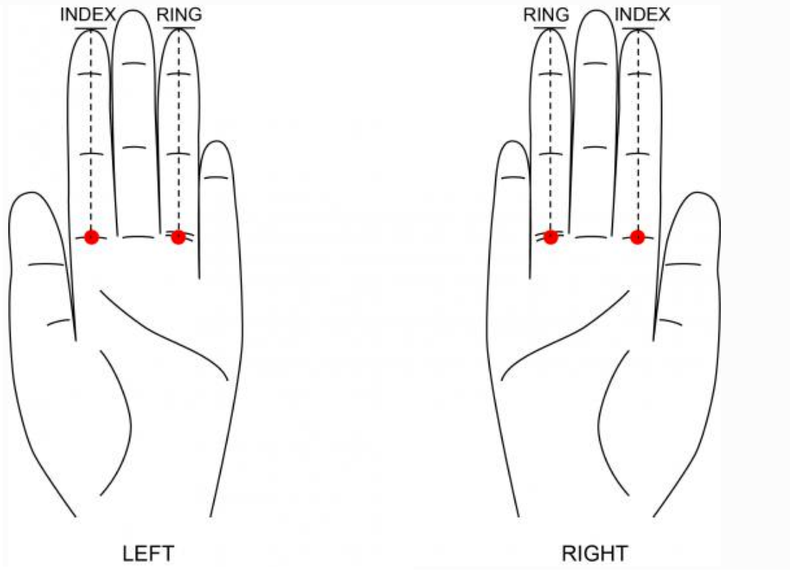
HYPOTHESIS

The ratio of the length of the index finger (2D) to the ring finger (4D) (2D:4D) has been reported to be lower (i.e., 2D<4D) in people with ALS in comparison to controls. So might increased exposure to prenatal testosterone, which lowers this ratio, also be a risk factor for ALS?

To test this hypothesis, we examined 2D:4Ds from large numbers of ALS respondents and controls.

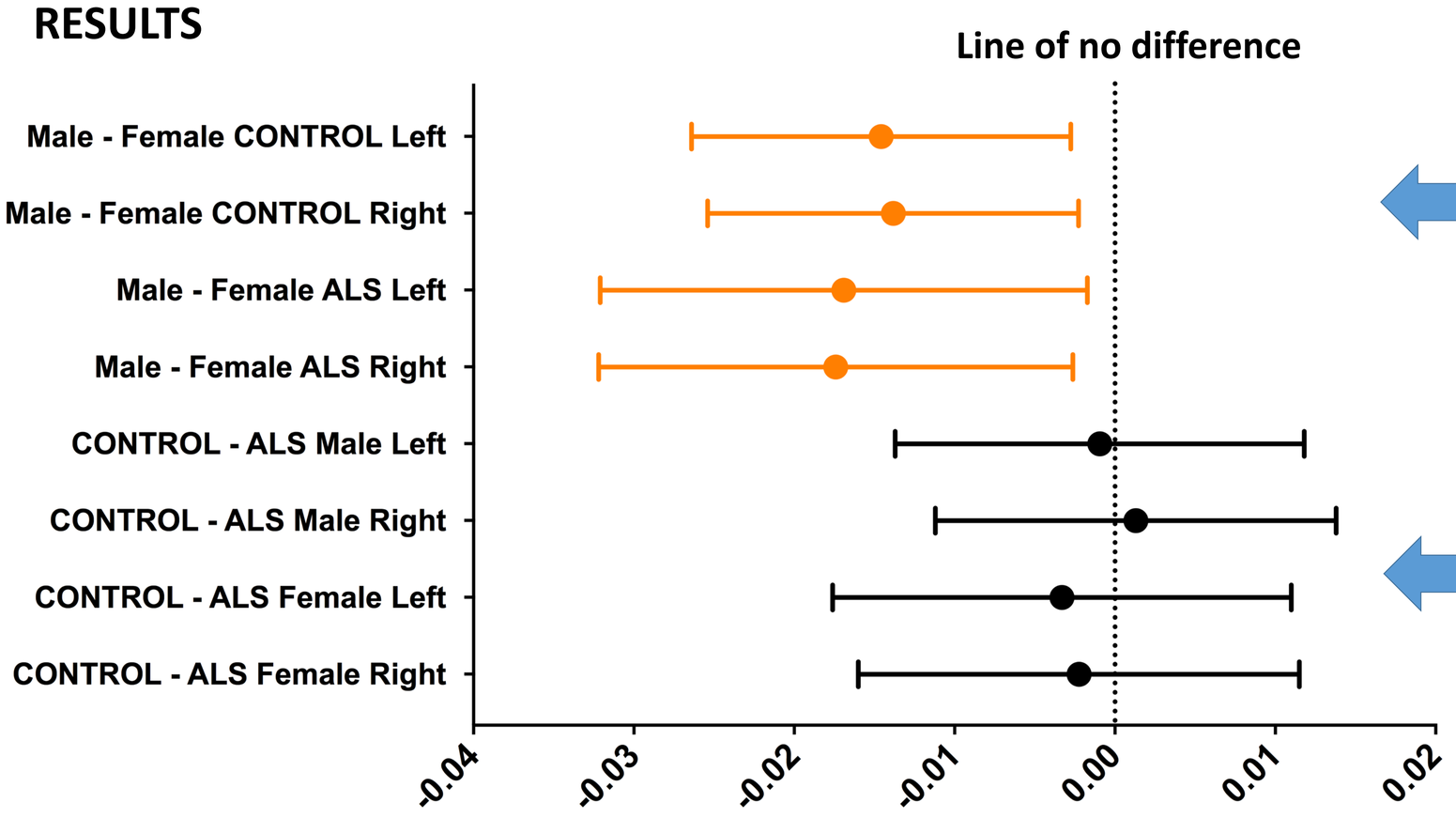
METHODS

- Responses were collected via a web-based questionnaire. Respondents self-measured and self-reported the lengths of their index and ring fingers on their left and right hands
- 202 ALS respondents (125 males, 77 females) and 370 controls (112 males, 258 females) remained after filtering for inability to straighten fingers, statistical outliers, and age <40 years
- Unpaired t-tests with 95% confidence intervals were used to assess differences in mean 2D:4Ds



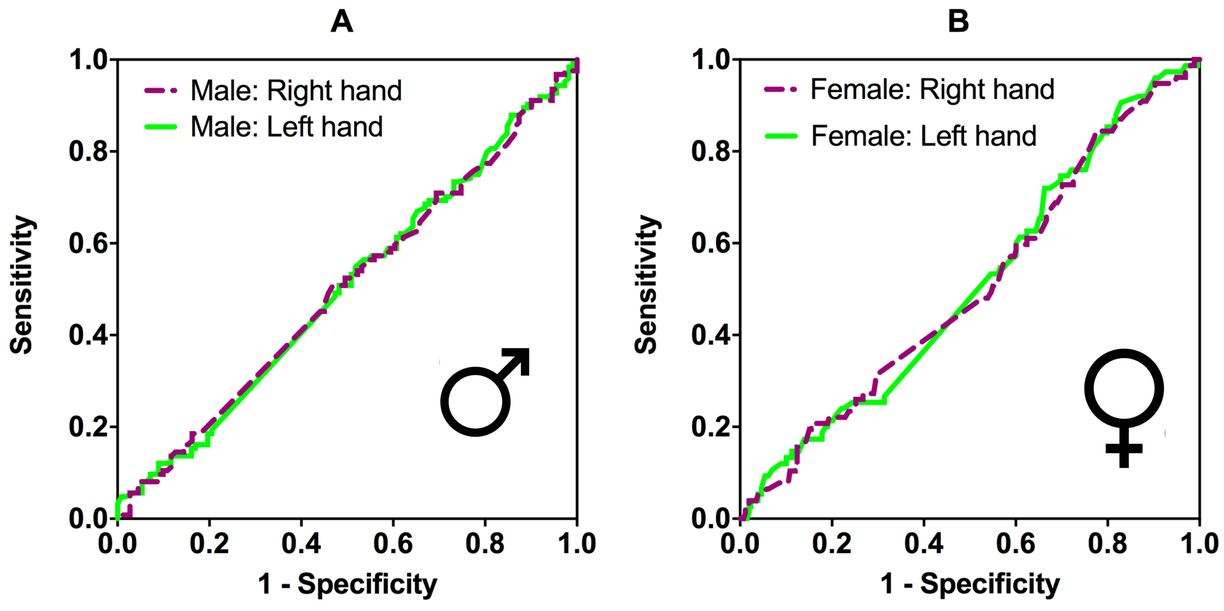
The diagram in the questionnaire to help respondents measure their finger lengths

RESULTS



Mean 2D:4Ds are smaller in *males* compared to *females*, in both control and ALS groups (orange bars). This gender difference is well established

No differences are seen in mean 2D:4Ds between *control* and *ALS* respondents, for either males or females (black bars)



ROC curves of 2D:4Ds in both males and females are close to the diagonal line of no-discrimination, indicating that the 2D:4Ds are neither sensitive nor specific to ALS status

CONCLUSIONS

- These findings do not support the hypothesis that exposure to increased prenatal testosterone is a risk factor for ALS
- A lower 2D:4D has been proposed to explain the link between ALS and exercise, but other exercise-related factors seem more likely to explain this association

Parkin Kullmann JA, Pamphlett R. Does the index-to-ring finger length ratio (2D:4D) differ in amyotrophic lateral sclerosis (ALS)? Results from an international online case-control study. *BMJ Open*. 2017;7:e016924.

APPENDIX G

Using an online tool for self-measurement and self-reporting of 2D and 4D finger lengths provides acceptable results: Findings from a validation study

USING AN ONLINE TOOL FOR SELF-MEASUREMENT AND SELF-REPORTING OF 2D AND 4D FINGER LENGTHS PROVIDES ACCEPTABLE RESULTS: FINDINGS FROM A VALIDATION STUDY

Jane Parkin Kullmann (JPK)

Roger Pamphlett (RP)

ABSTRACT

Background: The ratio of lengths between the index and ring fingers (the 2D:4D ratio) is increasingly being measured in neurological disorders to give an indication of intrauterine testosterone exposure, which may relate to the later appearance of disease. However, obtaining sufficient numbers of participants for statistical analyses, which is typically done via in-person recruiting, has been difficult. We therefore developed a web-based question that allows respondents to self-measure their finger lengths, but the reliability of these measurements needed to be assessed.

New Method: Volunteers were given a printed copy of the question from the online questionnaire which asked them to measure and record their index and ring finger lengths in millimeters. Digital photographs of their hands were taken and then used to measure their fingers again on a computer screen by two researchers. 2D:4D ratios were calculated using participants' and researchers' measurements and compared using paired t-tests. Inter-class correlation coefficients were also calculated for the researchers' ratios.

Results: A consistent difference of approximately 0.02 (all p-values <0.001) was observed between the ratios calculated using participants' and researchers' measurements, indicating a high but consistent bias for participants. Differences were not markedly dissimilar for the left and right hands. Inter-rater reliability between researchers was excellent.

Comparison with Existing Method(s): Research on the accuracy of self-measurement and self-reporting of finger lengths has typically compared self-measurements with measurements from photocopies; however, the use of photocopies has limitations. Using photographs, we have demonstrated that self-report of finger measurements is a valid approach.

Conclusions: Our study shows that self-report of finger lengths is an acceptably valid data-gathering approach for calculating 2D:4D ratios.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a progressive fatal neurodegenerative disease with a median survival time of approximately three to five years [1]. While the etiology of the disease is largely unknown, one element that has been suggested as a potential risk factor is prenatal exposure to testosterone [2]. Exposure to testosterone in utero is assessed by calculating the ratio of the lengths of the index (2D) and ring (4D) fingers on each hand (i.e., the 2D:4D ratio), where a lower 2D:4D ratio (i.e., a longer ring finger relative to the index) indicates higher prenatal exposure to testosterone [3,4].

However, obtaining sufficient numbers of participants for statistical analyses, which is typically done via in-person recruiting, has been difficult. Therefore, in our international web-based questionnaire of potential risk factors for ALS [5], we have included a question wherein respondents are asked to self-measure and self-report their 2D and 4D finger lengths on each hand. The measurements of finger lengths can be used to calculate a 2D:4D ratio for each hand of the respondent, and then the ratios for ALS patients and controls can be compared to evaluate the potential link between prenatal testosterone exposure and ALS.

Research on the accuracy of self-measurement and self-reporting of finger lengths has typically compared self-measurements with measurements from photocopies; however, the use of photocopies has limitations [6,7]. Therefore, we performed a validation study of the process of reporting self-measurements of 2D and 4D finger lengths, using photographs rather than photocopies, which also served to test the validity of the diagram created to show participants how to perform these measurements (Figure 1).

[Figure 1]

METHODS

Adult volunteers from Rotary International Clubs were recruited as participants for the validation study (N=131) throughout 2015. Participation was strictly voluntary, and participants completed the measuring task as part of a regular meeting of their Rotary Club. At each meeting, a presentation was given to the participants summarising the web-based ALS questionnaire and the objectives of the epidemiological study. This was done to provide a level of understanding of the project that would be equivalent to that of the respondents that would be filling out the questionnaire. Afterwards, participants were provided with a hard plastic ruler and a paper handout that is an exact replica of the instructions and diagram from the finger length question of the web-based questionnaire (Figure 1). Prior to distribution, the handouts were labeled with a unique alphanumeric code for each participant, so that each handout could later be linked with its associated digital photograph.

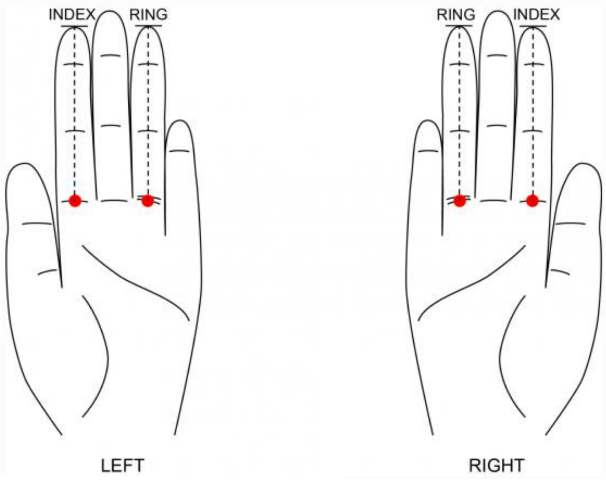
Figure 1. Instructions for Finger Measurement Question

Please measure the lengths of your index (second) and ring (fourth) fingers on each hand. The diagram below shows you how to make the measurements.

If you can only straighten out the fingers on one hand, only record the measurements for that hand.

If possible, please take off any rings prior to measuring your fingers.

First lay the back of your hand out flat on a level surface, with your fingers together. Then, with a rigid millimetre ruler (not a tape measure), measure from (1) the tip of the finger to (2) the middle of the crease at the bottom of the finger. The ring finger often has two of these creases, so here measure from the crease that is closest to the wrist.



Participants were asked to measure the 2D and 4D fingers on both hands. However, if they had an injury to one or both fingers on a hand, they did not record measurements for that hand. To best approximate the experience of the questionnaire, no questions were allowed and no further instructions (beyond the handout) were given while participants performed the task. The participants recorded their finger measurements directly on the handout. After completing the measurements, a digital photograph was taken of the underside of their hands overlaid on top of their handout (matching the layout of the hand image on the handout), so that the picture contained the code associated with their handout.

The self-reported measurements were entered into a Microsoft Excel spreadsheet, and identified by the participant code. 2D:4D ratios for each hand were calculated by dividing the length of the 2D finger on a given hand by the length of the 4D finger on the same hand.

Two researchers (JPK and RP) viewed the digital photographs on a computer screen and from there measured the lengths of the 2D and 4D fingers of the participants' hands with the same type of ruler used by the participants. Two participants were excluded due to poor photo quality. The researchers viewed the photos and recorded their measurements independently. 2D:4D ratios were then calculated for each hand of each participant using each of the researchers' measurements. Because the measurements were used to calculate a ratio and were not used as absolute values, it was not relevant what size the pictures were on-screen when measured by the researchers. Raw data for the validation study are provided in the Supplemental Material.

To compare the 2D:4D ratios calculated from the participants' and researchers' measurements, paired t-tests were conducted using the participants' ratios and the researchers' ratios for each hand (i.e., participant left/JPK left, participant left/RP left, participant right/JPK right, participant right/RP right). To evaluate the inter-rater reliability of JPK and RP, absolute two-way mixed single measure inter-class correlation coefficients (ICCs) were also calculated, pursuant to recommendations from the literature [8-10]. Statistics were calculated using IBM SPSS Statistics for Macintosh, Version 22.0.

RESULTS

Paired t-tests comparing the 2D:4D ratios calculated from the participants' and researchers' measurements show that a consistent mean paired difference of approximately 0.020-0.026 was observed between the ratios for the participants and the researchers, indicating a consistently high bias in the ratios calculated using the participants' measurements. Results for the left and right hands were similar, so measurements of each of the hands were considered to be of similar accuracy. The finger ratios, mean differences between the ratios, 95th confidence intervals, t-values, post-hoc power values, and effect sizes are summarised in Table 1. P-values for these results are <0.001.

Table 1. Summary of 2D:4D ratios and paired sample t-test results for the validation study.

Comparison Evaluated		Ratio Means		Paired Differences					
		Resp Mean (SD)	JPK/RP Mean (SD)	Mean Difference (SD)	95% Confidence Interval of the Difference		t-value	Power	Effect Size
					Lower	Upper			
Pair 1	Resp Left Ratio - JPK Left Ratio	0.988 (0.0424)	0.968 (0.0368)	0.0198 (0.0465)	0.0117	0.0279	4.83	0.998	0.426
Pair 2	Resp Left Ratio - RP Left Ratio	0.988 (0.0424)	0.968 (0.0464)	0.0204 (0.0573)	0.0104	0.0304	4.04	0.980	0.356
Pair 3	Resp Right Ratio - JPK Right Ratio	0.986 (0.0436)	0.966 (0.0355)	0.0199 (0.0346)	0.0122	0.0275	5.14	>0.999	0.575
Pair 4	Resp Right Ratio - RP Right Ratio	0.986 (0.0436)	0.960 (0.0417)	0.0262 (0.0446)	0.0184	0.0340	6.61	>0.999	0.587

Inter-rater reliability between researchers JPK and RP was high: the ICC for the left hand 2D:4D ratio was 0.784, and the ICC for the right hand 2D: 4D ratio was 0.796, indicating excellent agreement [11].

DISCUSSION

The results of comparisons between participants' and researchers' 2D:4D ratios are similar to those reported by Caswell and Manning [7]. For our study, we found that the differences in the ratios between the participants and the researchers are quite consistent, which could indicate that participants are generally under-measuring their 4D (ring) finger, perhaps by measuring from the higher of the two creases that are typically present on this finger (see Figure 1 for an illustration of the creases), or over-measuring their index finger. It has also been suggested that this difference may be attributable to the rendering of a three-dimensional object into a two-dimensional image, which would

result in a consistent difference between self-made measurements and measurements from a photo or photocopy [7].

Overall, there is reasonably good agreement between the participants' and researchers' ratios, which demonstrates the reproducibility and accuracy of the measurements being used to support the analysis of the data collected as a part of the case-control study. Further, the ICCs of the two researchers' ratios show excellent agreement (comparable to ICCs observed in a similar study [8]).

Comparison to Prior Work

The results of our validation study are very similar to those in the literature [7], although the prior study used photocopies rather than photographs. However, it is possible that the use of two-dimensional images, whether photocopies or photographs, has the same effect on viewers' perceptions of the fingers, the resulting measurements of finger lengths, and the calculated 2D:4D ratios.

CONCLUSIONS

Our validation study shows that self-measurement and self-report of 2D and 4D finger lengths is an acceptably valid approach for obtaining this information in a web-based questionnaire for use in calculating 2D:4D finger ratios.

ACKNOWLEDGEMENTS

We thank all of those who volunteered to participate in the validation study: Rotary Club of Burwood, Rotary Club of Drummoyne, Rotary Club of Five Dock, Rotary Club of the Inner West, Rotary Club of Maroubra, Rotary Club of Nashua (New Hampshire, USA), Rotary Club of Randwick, and Rotary Club of Ryde.

CONTRIBUTORSHIP STATEMENT

JPK analysed the results of the study and prepared the manuscript. JPK and RP conducted the validation experiment. RP reviewed the manuscript.

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APPENDIX H

Are people with ALS really “nice”?

(poster presentation at the International MND Symposium, 2017)

ARE PEOPLE WITH ALS REALLY "NICE"?

Jane Parkin Kullmann and Roger Pamphlett

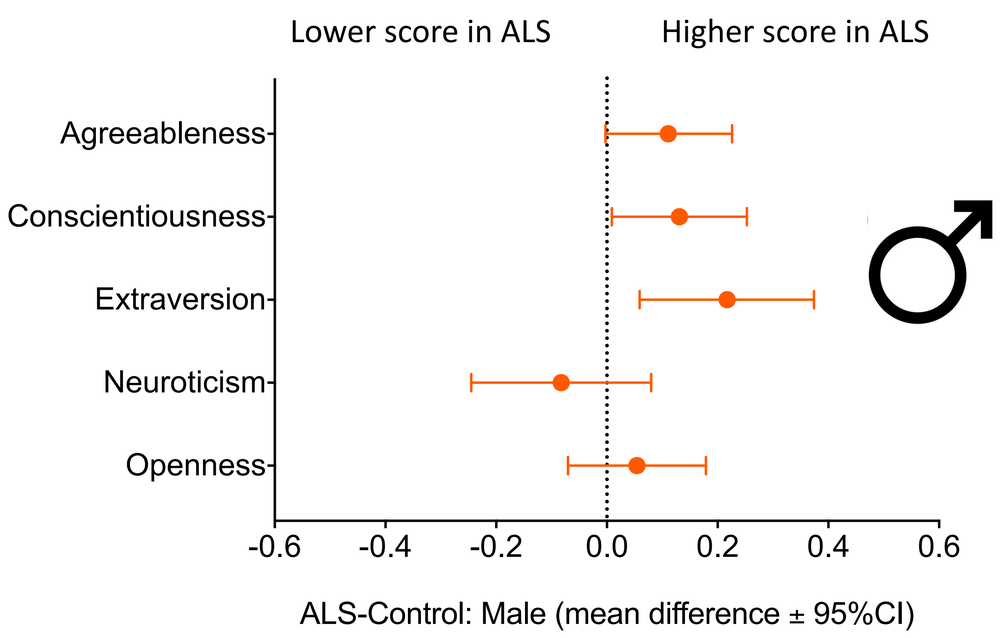
INTRODUCTION

- It has been suggested that people with ALS have a particularly "nice" personality
- Previous personality studies in ALS have had small numbers of subjects and conflicting results
- We used an online questionnaire to collect personality data from large numbers of people with ALS and controls

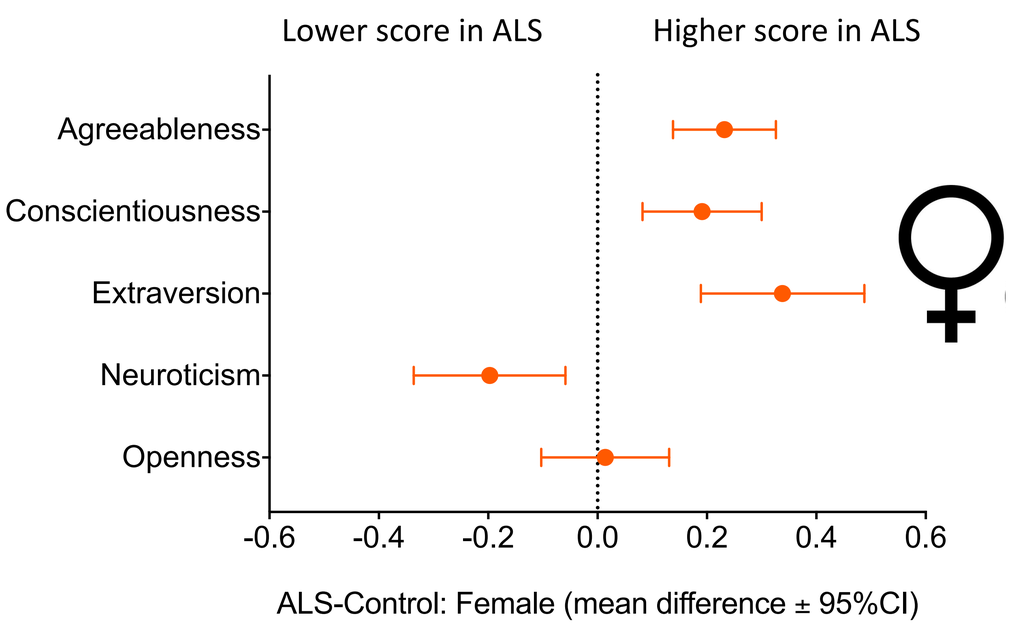
METHODS

- Responses to the 44 Big Five personality statements (agree or disagree, on a scale of 1 to 5) were collected via the web-based questionnaire "ALS Quest"
- Respondents were 339 people with ALS (212 male, 127 female) and 402 controls (120 male, 282 female), aged 40 years and over
- People with ALS were asked to respond to the statements as they applied to them *before* their diagnosis

RESULTS

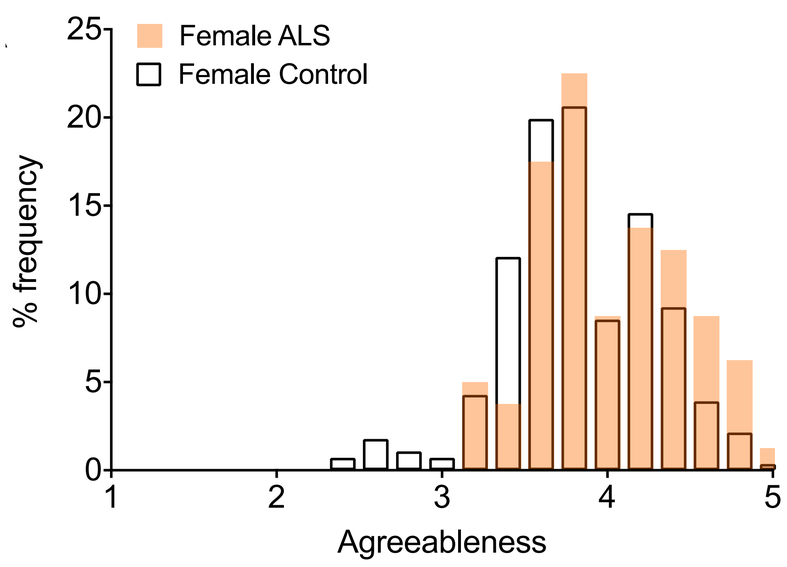


Male ALS respondents had higher mean scores for Conscientiousness and Extraversion

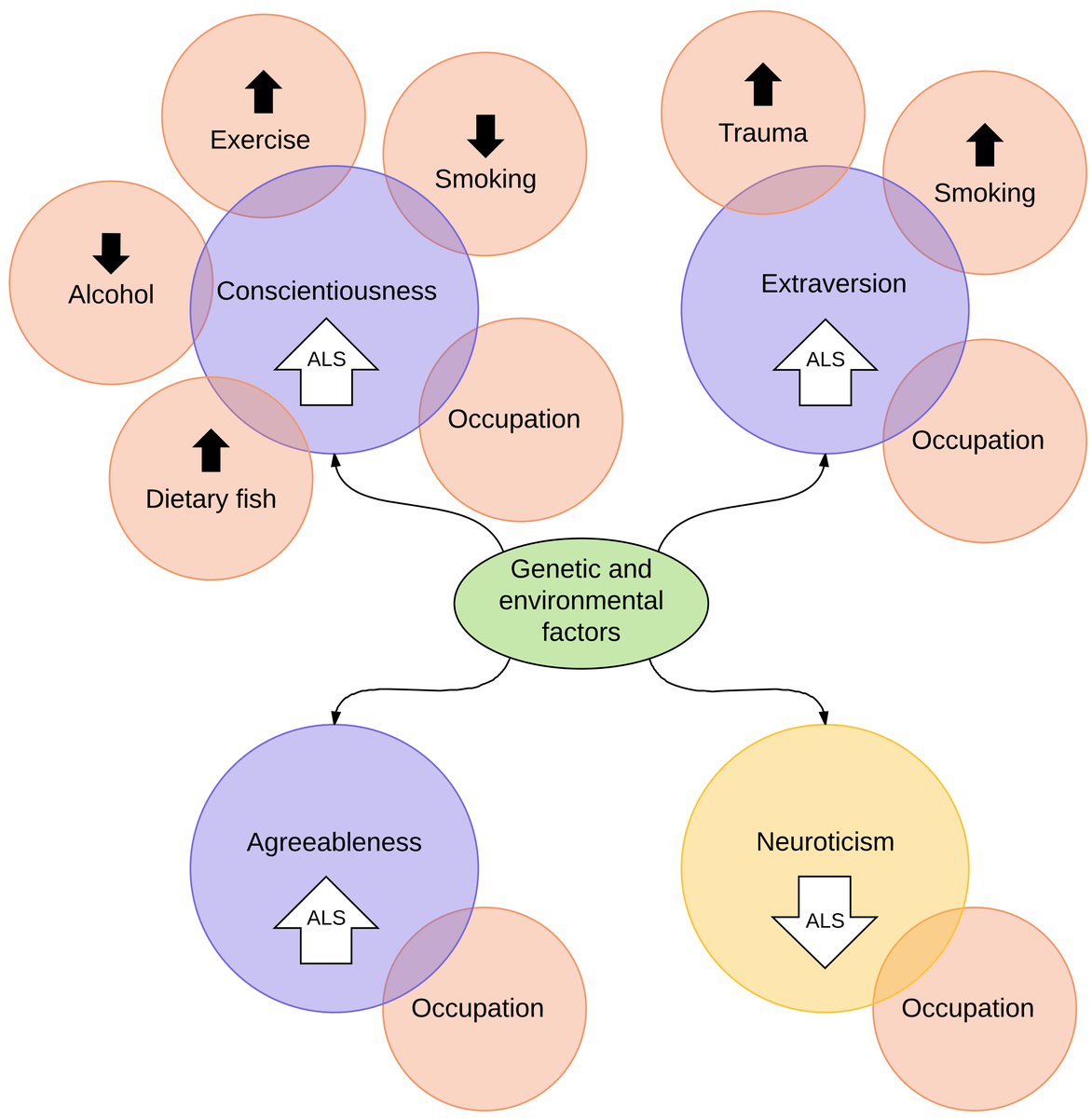


Female ALS respondents had higher mean scores for Agreeableness, Conscientiousness, and Extraversion, and a lower score for Neuroticism

The frequency distribution of personality scores overlapped for all factors. For example, here the distribution of Agreeableness scores for female ALS respondents is shifted to the right, but with overlap



PERSONALITY ASSOCIATIONS WITH ALS RISK FACTORS



Increased *Conscientiousness* in ALS could be associated with increased exercise, decreased alcohol intake, and increased fish consumption. Increased *Extraversion* could be associated with increased smoking, and risk-taking behavior leading to trauma. All factors could influence choices of occupations.

CONCLUSIONS AND RECOMMENDATIONS

- People with ALS as a group have personality traits that are likely to underlie the perception they are particularly "nice"
- ALS-associated personality traits could influence lifestyle choices, a number of which are suggested risk factors for ALS
- Personality testing should be included in future risk factor studies of ALS

APPENDIX I

Is psychological stress a predisposing factor for ALS? An online international case-control study of premorbid stressful life events, resilience and anxiety

(platform presentation at the International MND Symposium, 2018)

Is psychological stress a predisposing factor for ALS?

An online international case-control study of premorbid stressful life events, resilience and anxiety

SYDNEY MEDICAL SCHOOL

THE UNIVERSITY OF SYDNEY

JANE PARKIN KULLMANN
NEUROPATHOLOGY | BRAIN AND MIND CENTRE

THE UNIVERSITY OF SYDNEY

Background and Aims

- Stress is frequently mentioned as a possible disease trigger by ALS patients (29% in our survey)
- Stress has been implicated as a risk factor for other neurodegenerative diseases
- Stress could relate to ALS in the following ways:
  - Increased free radical activity
  - Reduced telomerase activity and telomere shortening due to early life stress
- In ALS, only one study (Okamoto et al. Ann Epidemiol 2009) has been published that looked at stress on a dichotomous basis (high/low)
- Aim of current study was to evaluate measures of stress along with modifying factors to look for a potential relationship between stress and ALS

Methods – ALS Quest Risk Factor Questionnaire

ALS QUEST

A QUESTIONNAIRE FOR RESEARCH INTO AMYOTROPHIC LATERAL SCLEROSIS AND MOTOR NEURON DISEASE

Welcome to ALS Quest.

ALS Quest is a research questionnaire developed by Doctor Roger Pamphlett at the University of Sydney to search for causes of amyotrophic lateral sclerosis.

We hope this project will find methods to prevent and to treat amyotrophic lateral sclerosis.

People with amyotrophic lateral sclerosis are invited to complete the questionnaire.

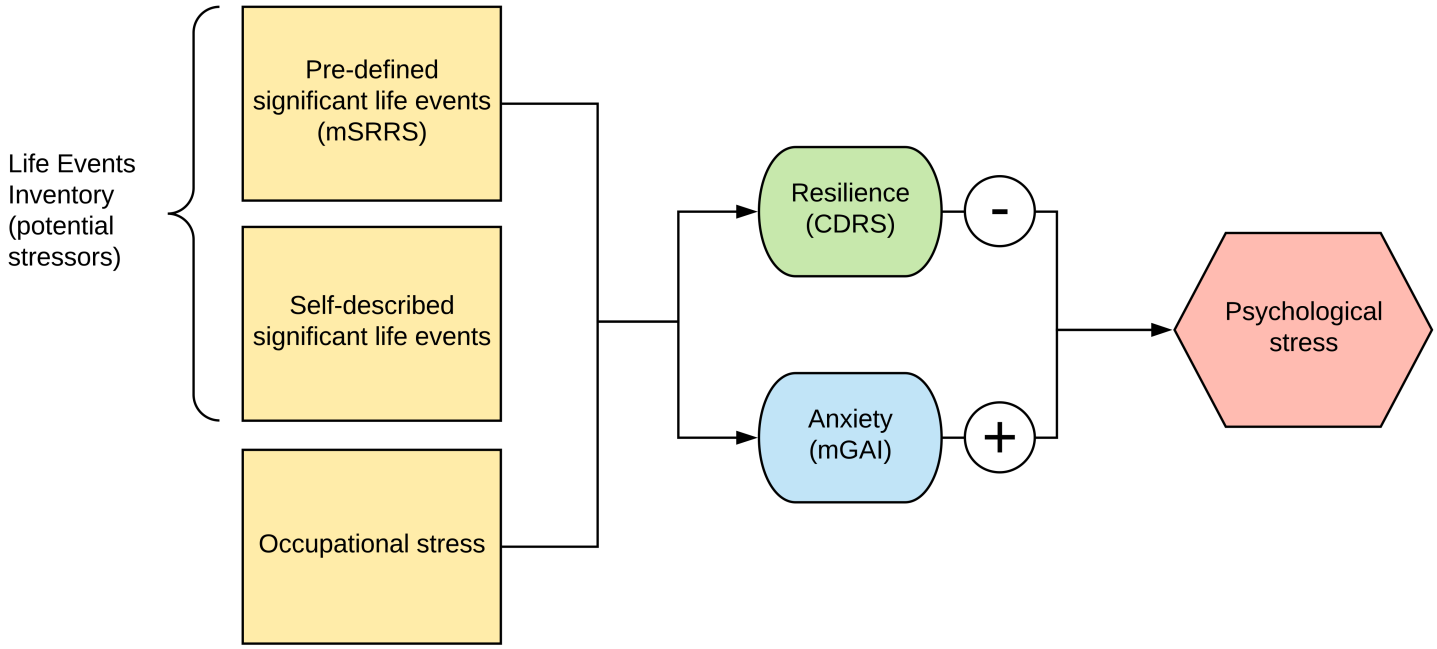
If you do not have amyotrophic lateral sclerosis, you are also invited to complete the questionnaire.

You will not be asked for any identifiable information.

Please select your language below and then click on the next button (>>) to begin or continue the questionnaire.

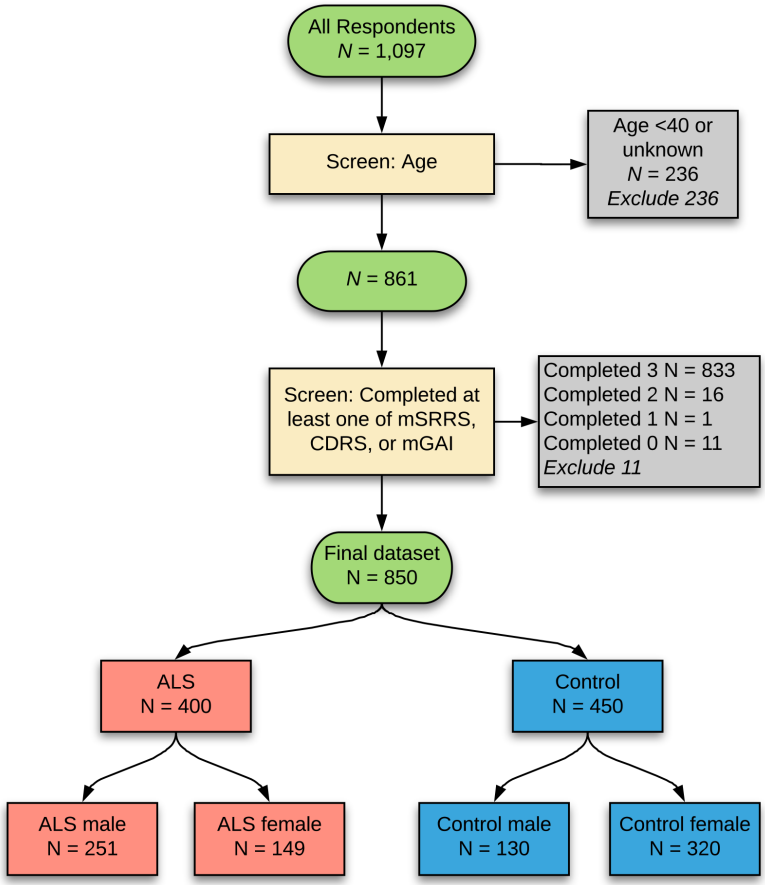
www.alsquest.org

Methods – Stress-Related Measurements



mSRRS: modified Social Readjustment Rating Scale
CDRS: Connor-Davidson Resilience Scale
mGAI: modified Geriatric Anxiety Inventory

Results – Respondents



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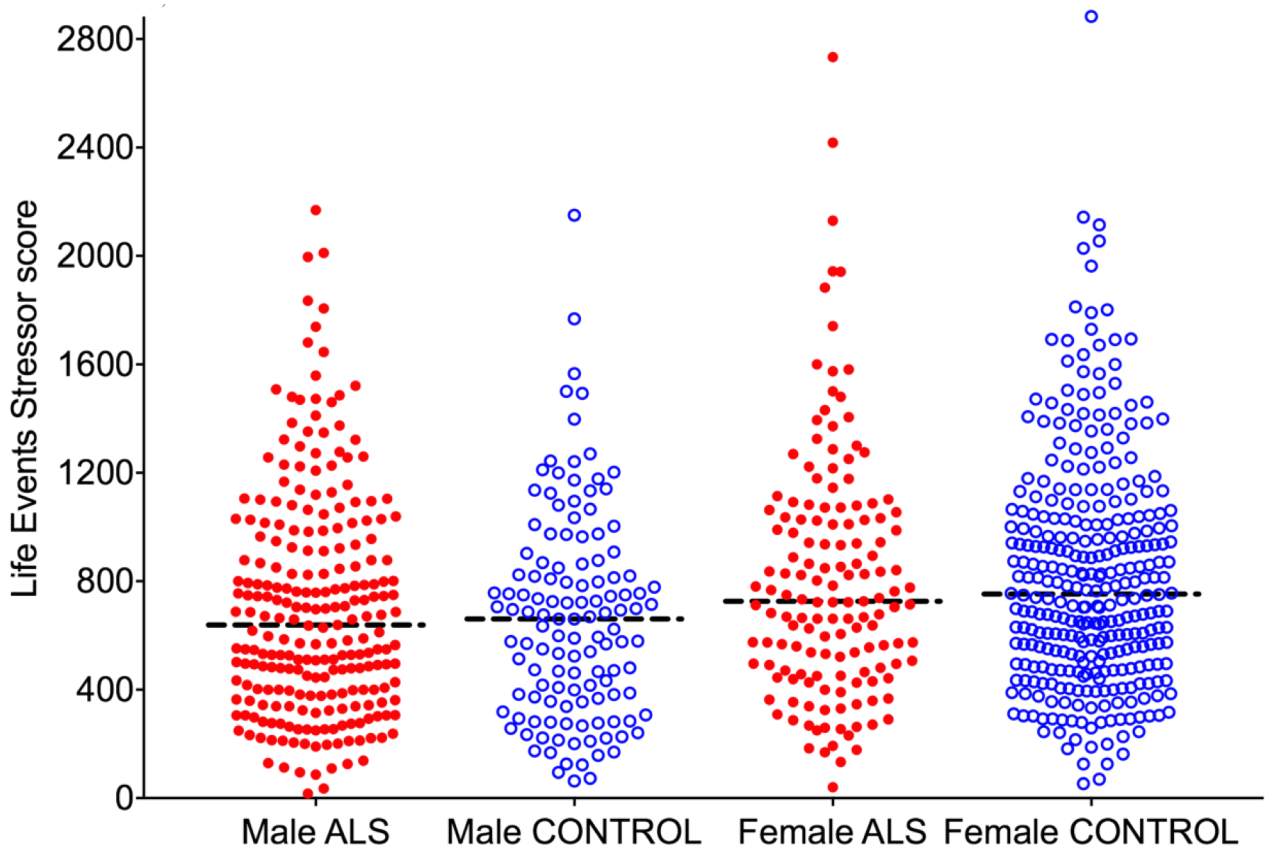
Results – Preliminary

- Factors evaluated:
  - Life Events: all, age 0-20 years, age 21-40 years, prior 2 years, prior 5 years, prior 10 years
  - Occupational Stress
  - Resilience
  - Anxiety

- Non-parametric statistics were used due to non-normality of data

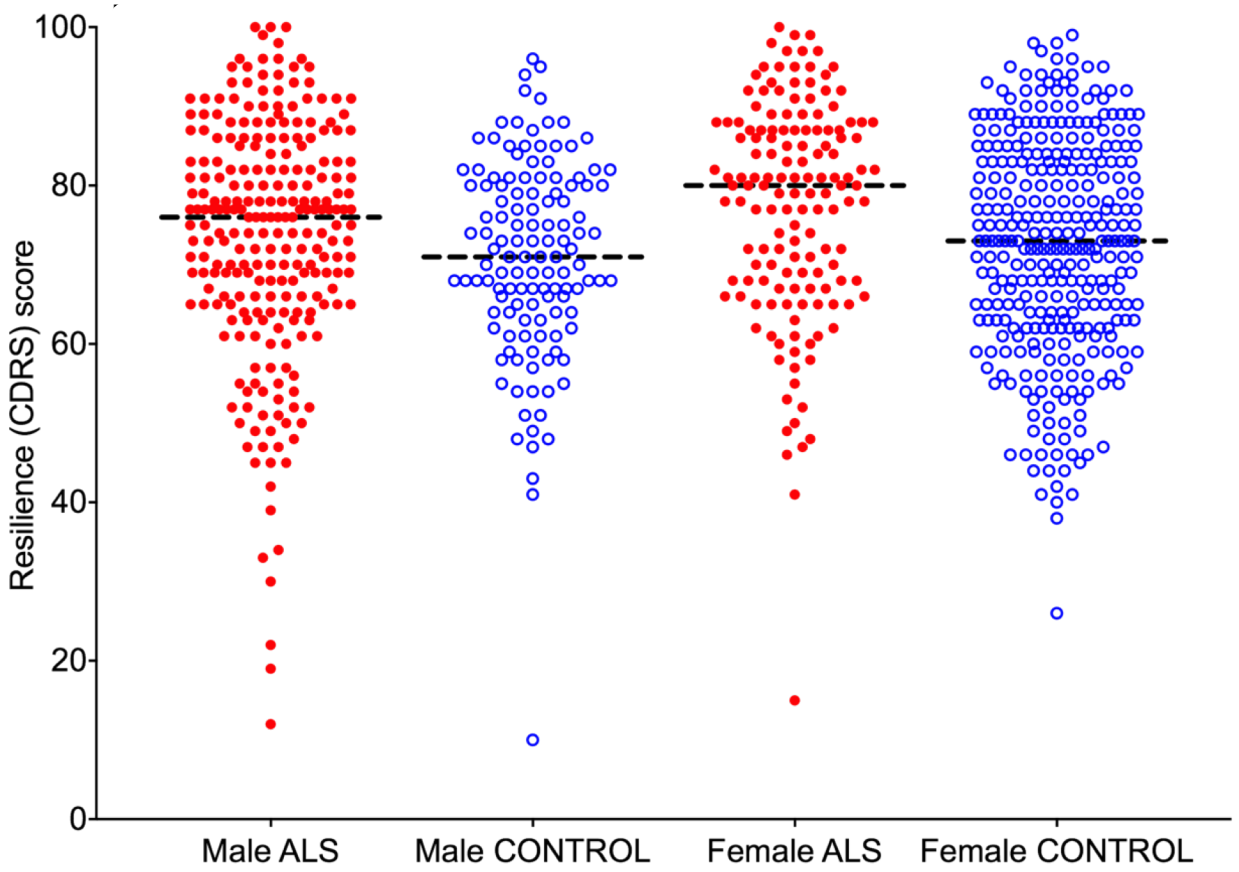
- Male-Female comparisons:
  - All were statistically significant, except for resilience
  - Further evaluations were performed separated by gender

Results – Life Events Stressor Score



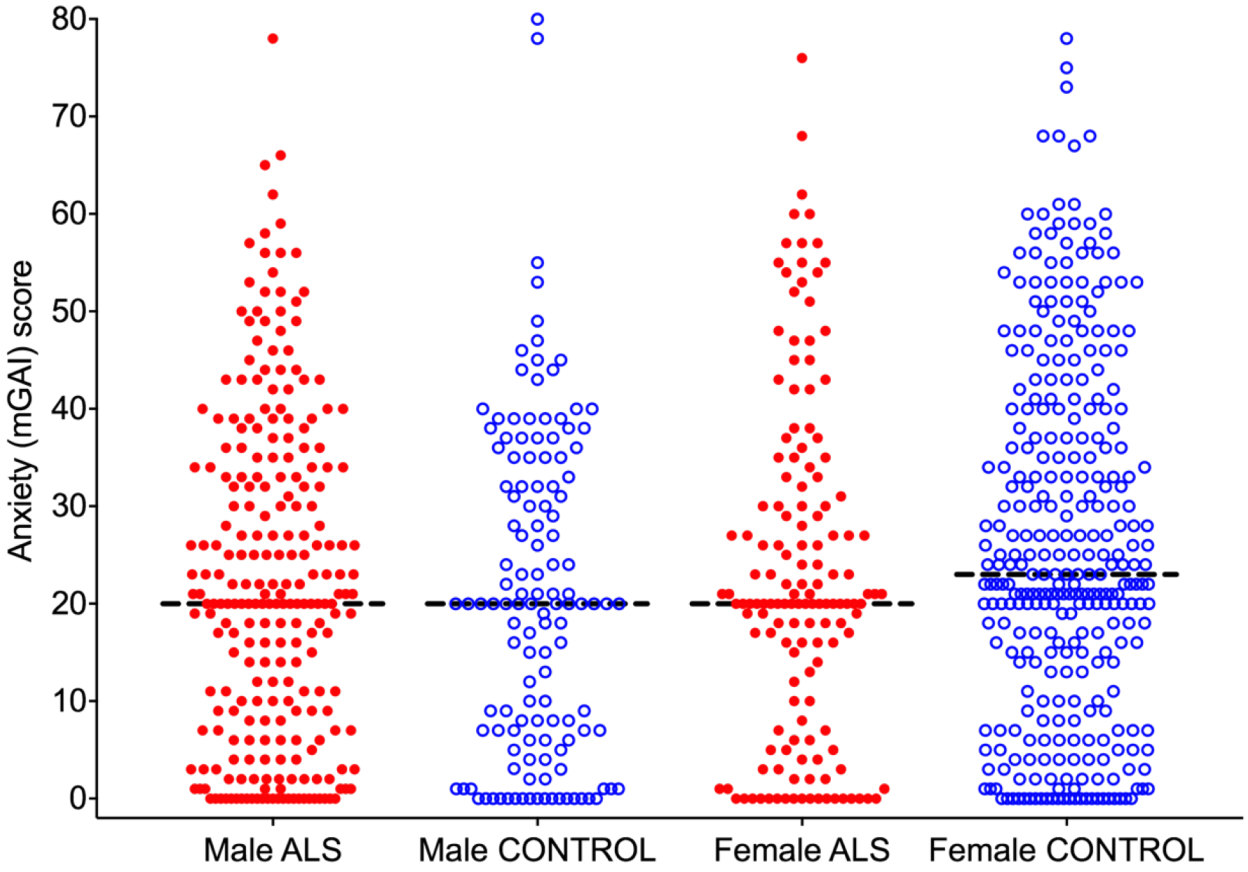
Parkin Kullmann JA et al (2018) PLoS ONE 13(9): e0204424

Results – Resilience



Parkin Kullmann JA et al (2018) PLoS ONE 13(9): e0204424

Results – Anxiety



Parkin Kullmann JA et al (2018) PLoS ONE 13(9): e0204424

Results – ALS vs. Controls

- Life Events Stressor Score:
  - No significant differences between male ALS respondents and male controls
  - Female ALS respondents had *lower* life events scores for the prior 5 years and prior 10 years in comparison to controls (p=0.01 and p=0.018)
- Occupational Stress:
  - No significant differences
- Resilience:
  - For both males and females, ALS respondents had *higher* resilience than their respective controls (p=0.028 and p<0.001)
- Anxiety:
  - No significant differences

Conclusions

- Stress does not appear to be a risk factor for ALS
- ALS respondents have higher resilience, which implies a more robust coping response to life stressors
- Resilience is thought to arise from a combination of factors that are genetic or biological, social or environmental, and personality-related
- Resilience may have genetic variants in common with ALS
- Resilience is also linked to greater conscientiousness, higher extraversion, and lower neuroticism, personality types that are more common in ALS (Parkin Kullmann JA et al Brain Behav 2018; e01119)

Acknowledgements

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- Questionnaire resources:
  - Stanford University ALS Consortium of Epidemiologic Studies (ACES)
  - European Amyotrophic Lateral Sclerosis Consortium (EURALS)
  - European Multidisciplinary ALS Network Identification to Cure Motor Neuron Degeneration (Euro-MOTOR)
  - Physicians' Health Study
  - Retrospective Screening of Traumatic Brain Injury (RESTBI) Questionnaire
  - NSW Prostate Cancer Care and Outcomes Study
  - French BMAALS programme questionnaire
- Those who have supported us by disseminating the questionnaire:
  - ALS/MND Associations
  - International Alliance of ALS/MND Associations
  - US CDC ALS Registry
  - Canadian Neuromuscular Disease Registry
- Questionnaire respondents
- Questionnaire translators

The questionnaire is open to all at www.alsquest.org

APPENDIX J

Mercury exposure from seafood and dental fillings is similar in people with and without ALS

(poster presentation at the International MND Symposium, 2018)

MERCURY EXPOSURE FROM SEAFOOD AND DENTAL FILLINGS IS SIMILAR IN PEOPLE WITH AND WITHOUT ALS

THE UNIVERSITY OF SYDNEY

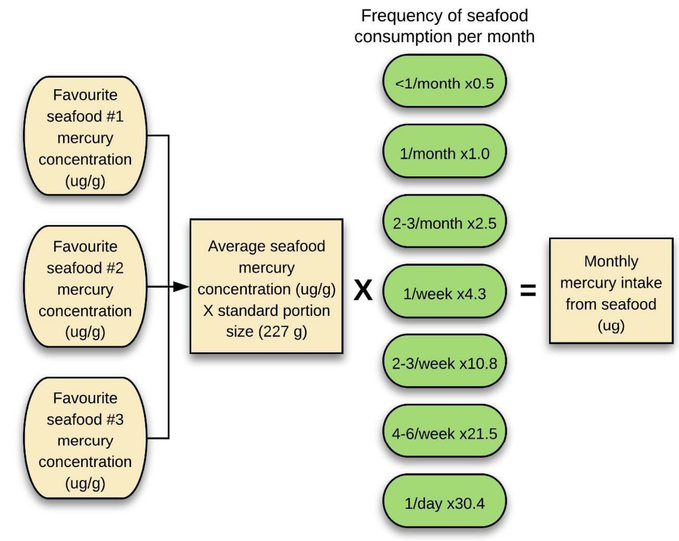
Jane Parkin Kullmann and Roger Pamphlett

INTRODUCTION

Exposures to toxic metals such as mercury have been suggested to be risk factors for ALS. Human intake of mercury commonly occurs via the consumption of seafood or from mercury-containing amalgam dental restorations (‘mercury fillings’). We therefore compared mercury exposures from these two sources in people with and without ALS.

METHODS

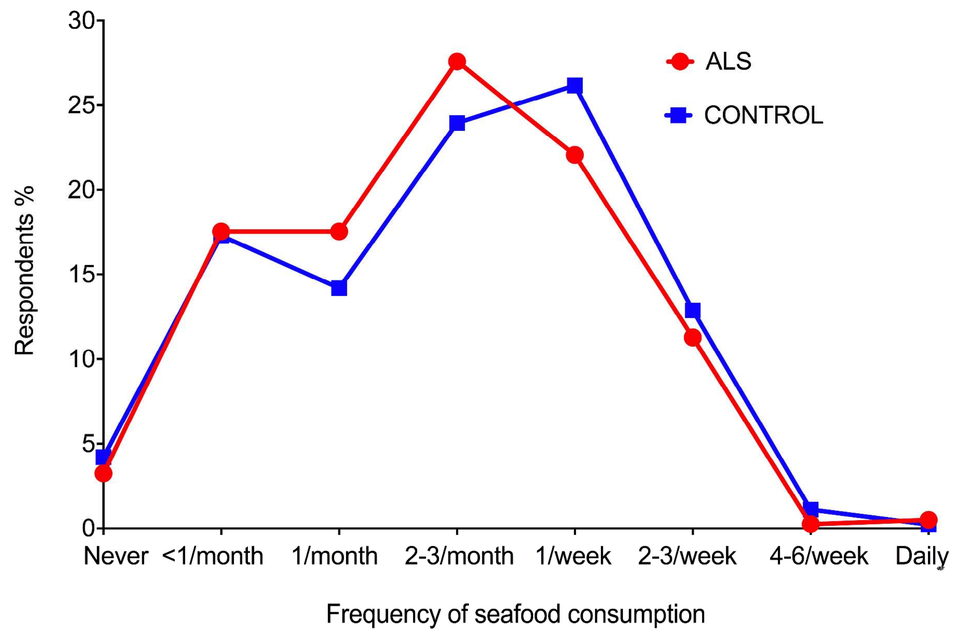
- Responses from people with ALS, as well as from non-ALS controls, were collected using an international multilingual online questionnaire looking for environmental and lifestyle risk factors for ALS (Designing an internationally accessible Web-based questionnaire to discover risk factors for amyotrophic lateral sclerosis: a case-control study. *JMIR Res Protos* 2015).
- Information collected from respondents included how often they ate seafood and what their favorite types of seafoods were.
- Mercury concentrations in favorite seafoods were averaged, multiplied by a standard seafood meal weight, and by the frequency of seafood consumption, to calculate monthly mercury exposure from eating seafood (Figure on right).
- Respondents were also asked to record how many current or former mercury-containing 'silver' amalgam dental fillings they had, and, if current, whether these were occlusal or non-occlusal.



Estimating mercury exposure from seafood consumption. The average mercury concentration in µg/g from up to three favorite seafoods was multiplied by a standard portion size of seafood (227 g), then multiplied by the monthly frequency of seafood consumption to estimate monthly mercury exposure from seafood in micrograms.

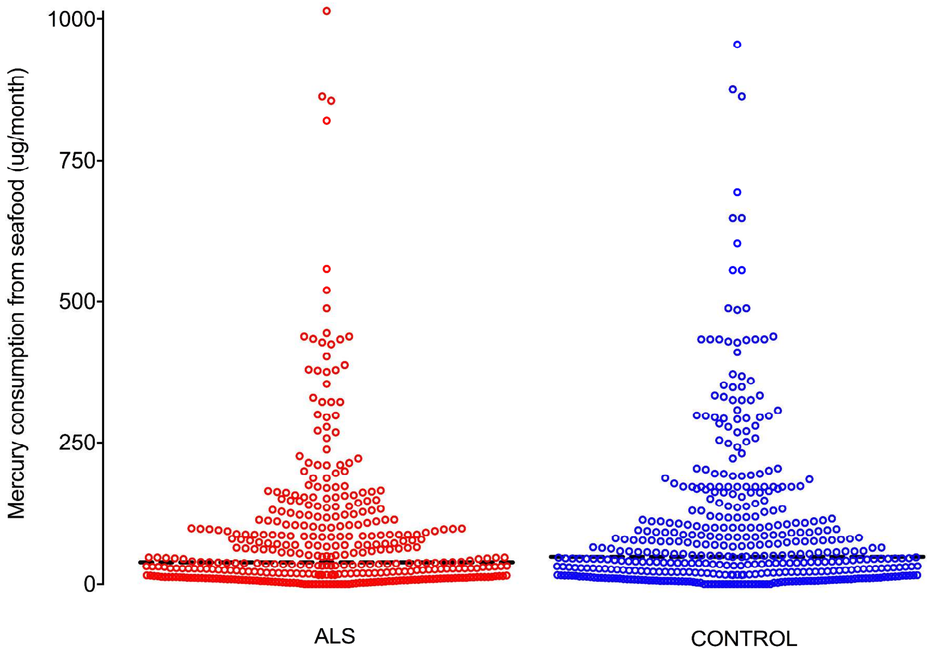
RESULTS

- Respondents aged 40 years and over were 401 with ALS (252 male, 149 female) and 452 non-ALS controls (130 male, 322 female).
- ALS respondents and controls did not differ in their frequency of seafood consumption (Figure below).



The proportion of respondents eating seafood at different frequencies. ALS and control respondents ate seafood at similar frequencies, ranging from never to daily. None of the slight differences in frequency proportions between ALS and controls was statistically significant.

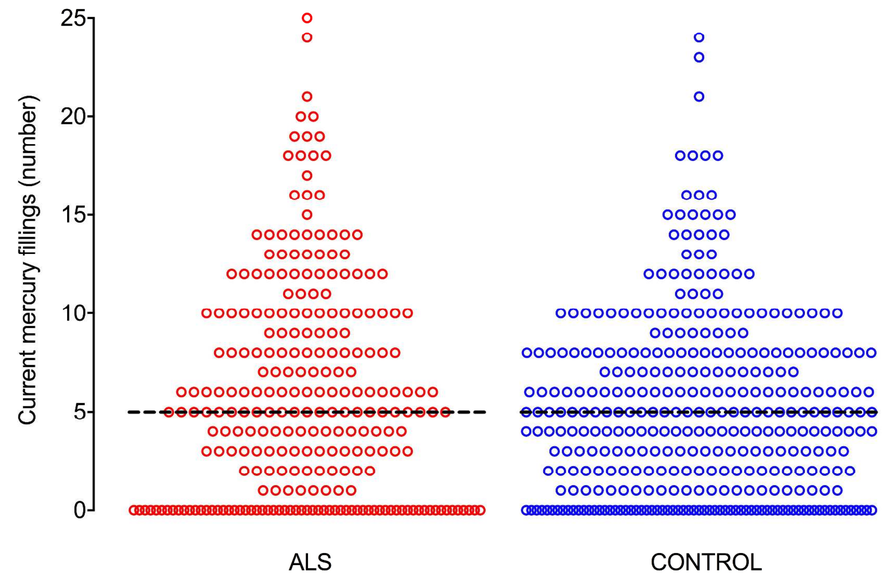
- ALS respondents and controls did not differ in the monthly mercury intake from their favorite seafoods (Figure below).



Distribution of mercury exposure from seafood. No difference is seen in the distribution of monthly mercury exposure from seafoods in micrograms between ALS and control respondents. Bar: median monthly exposure (µg).

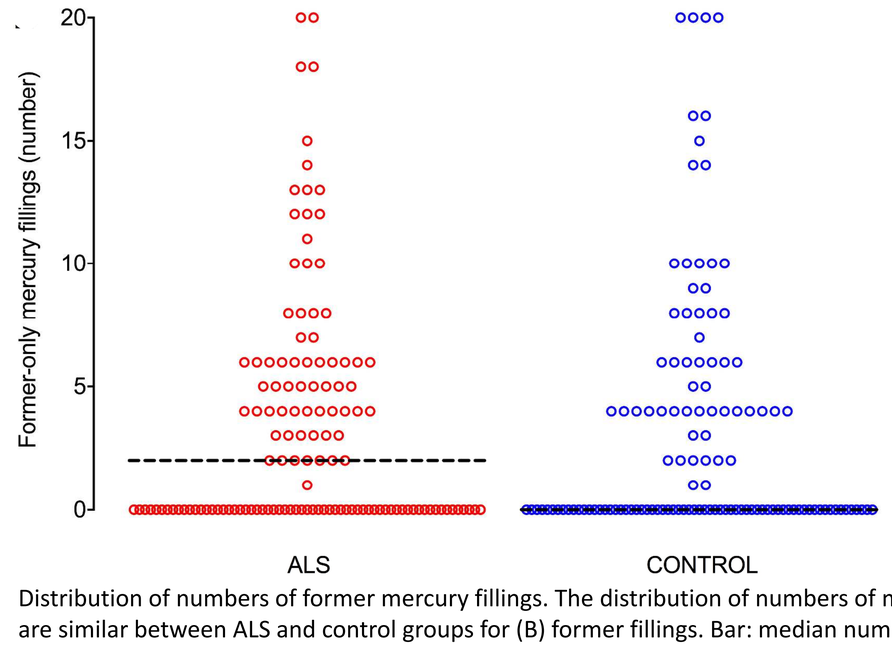
- Australians ate seafoods with higher mercury concentrations than USA respondents, but within individual countries no ALS-control differences were found in mercury seafood intake.
- ALS respondents ate more shellfish as a favorite seafood.

- ALS and control respondents had similar numbers of *current* combined occlusal and non-occlusal mercury fillings (Figure below).



Distribution of numbers of current mercury fillings. The distribution of numbers of mercury fillings are similar between ALS and control groups for (A) current fillings. Bar: median number of fillings.

- ALS and control respondents had similar numbers of *former* mercury fillings (Figure below).



Distribution of numbers of former mercury fillings. The distribution of numbers of mercury fillings are similar between ALS and control groups for (B) former fillings. Bar: median number of fillings.

- People with ALS were *less* likely than controls to have current occlusal mercury fillings.

CONCLUSIONS

- No evidence was found that mercury exposure from eating seafood or from having mercury-containing dental fillings was associated with the risk of developing ALS.
- If mercury does play a role in the pathogenesis of ALS, either:
  - i. Other sources of exposure to mercury in the environment or workplace need to be considered, or
  - ii. A particular susceptibility to mercury toxicity in ALS, such as genetic or epigenetic variations, multiple toxic metal interactions, or selenium deficiency, may be present.