UK Case control study of smoking and risk of Amyotrophic Lateral Sclerosis

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Sarah Opie-Martin is a PhD student and computer scientist working on the set up, data collection and analysis of epidemiology projects in amyotrophic lateral sclerosis. Her research focusses on environmental risk factors for ALS, clinical subgroups, and disease prognosis.

Ashley Jones is an MND Association Non-clinical Junior Fellow who specialises in integrative omics: uniting DNA, transcriptomics and methylation to understand the disease process of Amyotrophic Lateral Sclerosis and to facilitate drug discovery. His background is in statistics, bioinformatics, disease modelling, post-mortem transcriptomics and methylation, GWAS, network analyses and machine learning methods.

Alfredo Iacoangeli accepted his PhD "cum laude" in Life Sciences in January 2016 from "Sapienza" University of Rome where he worked in Tramontano's Lab on structural bioinformatics with a particular focus on protein structure prediction and protein-peptide interaction. In the same institution he also obtained a Bsc in physics and a 2 years Msc in physics of biosystems. He is now postdoctoral researcher in Dobson's group as part of a joint grant between the MRC Centre for Neurodegeneration Research, Department of Clinical Neuroscience (Professor Ammar Al-Chalabi) and the MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience (Professor Richard Dobson) whose aim is the development of a high throughput gene, environment and epigenetics database and analysis system for international ALS research. Dr. Iacoangeli is first author of several scientific articles in the field of Structural and Genomic Bioinformatics and ALS genetics.

Dr Ahmad Al Khleifat is a clinician and scientist currently working with Professor Al-Chalabi’s group. His main focus of research is on ALS clinical staging, clinical trial data analysis, and disease gene identification through next generation sequencing and coupling this with advanced data analysis to deliver diagnostic tools for complex disease genetics. Since 2017 Dr Al Khleifat has been a co-chair of genomic structural variations group responsible for the collection of the largest genomic single disease cohort in the world; Project MinE. Dr Al Khleifat is the leading the analysis for the structural variation genome wide association analysis using 10,500 samples from the Project MinE initiative.

Since joining King’s College London, Mohamed has been involved with studies related to medicine and theology. He recently completed an MSc in Clinical Neuroscience with a distinction. Mohamed is also a Co-Lead of the ARK Project at the university which is organizes volunteering opportunities for students at the university. These opportunities vary from feeding the homeless to helping the elderly in the community. He is also an active member of the PAL scheme which aims to help younger students with their academic studies.

Prof Dame Pamela Shaw (<http://sitran.org/people/shaw>) is an ALS neurologist and NIHR Senior Investigator. She is Director of the Sheffield Motor Neuron Disease Care and Research Centre, Director of the Sheffield Institute for Translational Neuroscience (SITraN), and Director of the NIHR Biomedical Research Centre Translational Neuroscience for Chronic Neurological Disorders. She has expertise in the biological mechanisms underlying ALS, and their role in determining disease progression and manifestation, as well as extensive experience in ALS clinical trials (h-index 92). She founded and previously chaired the UK MND Clinical Studies Group under the NIHR DeNDRoN network.

Christopher Shaw is Professor of Neurology and Neurogenetics at King’s College London. He is Director of the Maurice Wohl Clinical Neurosciences Institute and Deputy Director of the United Kingdom Dementia Research Institute at King’s College London.

He runs a clinic for people with motor neuron disorders at King’s College Hospital. His early clinical training was conducted in New Zealand and in 1992 he came to Cambridge on a Wellcome Trust Fellowship. Over the past 25 years his team have created one of the world’s largest Biobanks of DNA samples, lymphoblast cell lines and post mortem tissues from patients with MND. This has underpinned his research exploring the genetics, molecular and cellular pathobiology of MND and Frontotemporal Dementia.

His team have led the discovery of mutations in more MND genes than another other laboratory. They have also developed a large number of stem cell and transgenic mouse models of disease that have revealed important insights into disease mechanisms and novel drug targets. Their major focus is to advance gene therapies using antisense oligonucleotides and adenoviral gene vectors.

Karen Morrison is Associate Dean, Education, and Director of Education at the University of Southampton. She is also Professor of Neurology and an Honorary Consultant Neurologist, with expertise in the diagnosis and management of people with motor neurone disease. She graduated in Medicine from Cambridge and Oxford and, after postdoctoral research at Yale University, returned to Oxford, establishing a research programme in inherited disorders of motor nerves. She led clinical neuroscience research as the Bloomer Professor of Neurology at the University of Birmingham for 17 years, combining research into molecular genetic mechanisms in neurodegenerative disease (Parkinson’s disease and MND/ALS) with neurology/neuroscience teaching and co-directing the clinical MND Care and Research Centre there. As Associate Dean in Southampton, she leads Education in the Faculty of Medicine, ensuring that all graduates are equipped with the clinical, academic and professional skills to ensure successful careers in medicine and research as lifelong learners.

Dr Robyn Wootton is a genetic epidemiologist focused on health behaviours and mental health. My PhD at the University of Bristol investigated the genetic aetiology of subjective wellbeing and other positive mental health outcomes including optimism, gratitude and trust. Her current postdoctoral research applies the method of Mendelian Randomisation to understand the bi-directional relationship between health behaviours (e.g. smoking, alcohol and BMI) and mental health.

Professor George Davey Smith is a clinical epidemiologist and the director of the MRC Integrative Epidemiology Unit (IEU) at the Bristol Medical School, University of Bristol. His research has pioneered understanding of the causes and alleviation of health inequalities; lifecourse epidemiology; systematic reviewing of epidemiological evidence; and the study of population health contributions of the new genetics. He is particularly interested in developing and applying Mendelian randomization approaches, interrogating the causal role of behavioral factors (such as alcohol consumption) and intermediate phenotypes on different health outcomes, such as cardiovascular diseases and type 2 diabetes. He is currently Scientific Director of the Avon Longitudinal Study of Parents and Children.

Neil Pearce is Professor of Epidemiology and Biostatistics at the London School of Hygiene and Tropical Medicine. His current research interests focus on epidemiological and biostatistical methods, and their application to studies of non-communicable diseases (NCDs), including occupational and environmental health, asthma, kidney disease and neurological disease. He has a particular interest in global epidemiological studies, and was President of the International Epidemiological Association during 2008-2011.

Ammar Al-Chalabi is Professor of Neurology and Complex Disease Genetics at King’s College London, and Consultant Neurologist at King’s College Hospital, London, where he is Director of the King’s Motor Nerve Clinic. His clinical and research focus is amyotrophic lateral sclerosis (ALS). His team has developed a clinical staging system for ALS, and with others, a fundamental mechanistic hypothesis of ALS causation, showing it is likely a 6-step multistep process in which genetic factors may account for more than one step. He co-leads the international Project MinE consortium, sequencing more than 22,000 whole genomes, most from people with ALS, and with his team, has contributed to the identification of most known ALS genes.

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Introduction

Susceptibility to amyotrophic lateral sclerosis (ALS) is associated with smoking in some studies, but it is not clear which aspect of smoking behaviour is related. Using detailed records of lifetime smoking we investigated the relationship between smoking and ALS in a UK population.

Methods

In this retrospective case-control study, smoking status was collected using environmental questionnaires from people diagnosed with ALS between 2008 and 2013 and from age, sex and geographically matched controls. Categorical measures of smoking behaviour were: smoking at time of survey and smoking initiation; continuous measures were intensity (cigarettes per day), duration (years from starting to stopping or time of survey), cigarette pack years, and comprehensive smoking index (CSI), a measure of lifetime smoking. We used logistic regression to assess risk of ALS with different combinations of smoking variables adjusted for age at survey, gender, level of education, smoking status and alcohol initiation, selecting the best model using the Akaike Information Criterion.

Results

There were 388 records with full smoking history. The best fitting model used CSI and smoking status at time of survey. We found a weak association between current smoking and risk of ALS, OR 3.63 (95% CI 1.02-13.9) p-value 0.05. Increase in CSI score did not increase risk of ALS: OR 0.81 (95% CI 0.58-1.11) p-value 0.2.

Conclusion

There is weak evidence of a positive effect of current smoking on risk of ALS which does not show dose-dependence with higher levels of lifetime smoking and may be a false positive result.

**Introduction**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by progressive death of motor neurons leading to relentlessly worsening weakness and death, usually from respiratory failure due to involvement of the diaphragm, 2-3 years after diagnosis(1, 2) Although there is an evident genetic component, heritability studies indicate that environmental (and probably stochastic) factors also contribute (3-6).

There is evidence from multiple studies that smoking is associated with ALS, but no agreement over which aspect of smoking behaviour is related to ALS (7-14). Despite an evidence-based literature review that concluded that smoking can be considered a risk factor for ALS, it remains unclear if there is a dose-response effect, or what the biological mechanism might be (15). In addition, confounding cannot be discounted, since ALS is also associated with military service, education and socioeconomic status, which are also associated with smoking status (16, 17). It is biologically plausible that smoking could be a risk factor through oxidative stress or exposure to potentially neurotoxic chemicals, and so it remains an attractive candidate for studies of environmental aetiology (18, 19).

The comprehensive smoking index (CSI) estimates lifetime smoking by combining duration, intensity and time since cessation into a score allowing all factors to be considered while avoiding issues of multicollinearity between smoking exposure variables (20). CSI has not previously been used to investigate the role of smoking in ALS risk.

We therefore analysed retrospective case-control data to determine whether smoking is related to ALS in a UK population, investigating the relationship between different smoking variables including CSI and other regularly used measures, and risk of ALS.

**Methods**

***Case-control study design***

The data were obtained from the Motor Neurone Disease Association of England, Wales and Northern Ireland (MNDA) Collections collected as part of the MNDA Epidemiology Study, REC reference 07/MRE01/57. People diagnosed with definite, probable or possible ALS according to the El Escorial criteria between 2008 and 2013 were included (21). Three tertiary centres in London, Sheffield and Birmingham acted as data collection hubs but people with ALS were recruited at secondary centres such as district general hospitals, therefore these are incident cases representative of the ALS population. General practitioners from the general practice of the person with ALS were asked to invite 10 healthy controls to participate in the study via post. The research team matched people on age (within 5 years of the person with ALS) and gender in a 1:1 ratio. 413 participants provided informed consent, 405 undertook a telephone interview about their lifestyle including smoking undertaken by a trained nurse. 3 participants gave no information on smoking behaviour.

***Definition of smoking status***

Categorical measures were: smoking at time of survey (current, former, never), smoking initiation (ever, never).

To define former smokers we used logistic regression modelling to compare ALS risk between current smokers and ex-smokers, using never smokers as a reference. Few people had recently quit (n=3 within one year of survey) so we grouped ex-smokers into 5-year time since cessation intervals up to 20 years which was aggregated to 20+. ALS risk reduced from an odds ratio of 2.02 to 0.79 for current smokers compared to people who had quit within 5 years so former smokers were defined as having given up at least a day before the survey.

Continuous measures included: intensity (cigarettes per day), duration of smoking (years from starting to stopping or time of survey), pack years (intensity x duration), and CSI. The CSI is a non-linear model of smoking exposure that combines duration of smoking, time since cessation and smoking intensity into a continuous score which can be used in a regression model representing lifetime smoking (20). The model involves simulation of tau and delta from the dataset. Delta, or half-life, reflects the exponential decay in the effect of smoking on health outcomes during a lifetime. Tau, or lag-time, reflects that smokers may be at a higher risk of disease immediately after quitting due to reverse causality. The equations for CSI are as follows:

tsc\* = max(tsc - δ, 0)

dur\* = max(dur + tsc - δ, 0) – tsc\*

comprehensive smoking index = (1 – 0.5dur\*/τ) (0.5tsc\*/τ) ln(int+1)

tsc = time since cessation, δ = lag time, tss = time started smoking, dur = duration of smoking (calculated as age-tss for people currently smoking or [age-tsc]-tss for former smokers), τ = half-life, int = cigarettes per day.

***Logistic Regression***

Data were analysed using R. Continuous demographic characteristics were compared by Student’s *t*-Test or Mann-Whitney U test. Categorical variables were compared by chi-squared or Fisher’s exact test. The primary outcome, whether smoking increases risk of ALS, was analysed using logistic regression with maximum likelihood estimation. We generated 8 models with combinations of one categorical and one continuous measure of smoking, comparing the Akaike Information Criterion (AIC) of the models to assess fit (23). Odds ratios were adjusted for age, educational attainment, gender and alcohol consumption.

Assuming an odds ratio of 1.8, a 20% smoking rate in the control population and alpha of 0.05, we had 71% power with a sample size of 400 cases and controls in a 1:1 ratio.

**Results**

There were 202 cases and 200 control records available for analysis. The two groups were similar except for educational attainment and alcohol status. The details are shown in table 1. [Insert table 1 here]

The optimal CSI variables were tau = 2 and delta = 3.6. There were no differences between groups in unadjusted smoking behaviours, as shown in table 2. [Insert table 2 here]

388 records had full smoking history available for logistic regression analysis. Table 3 gives the results of the best fitting logistic regression model which included the CSI and smoking status at time of survey with AIC 543.77. The highest AIC, representing the worst fitting model, was for smoking initiation and number of cigarettes per day at 553.23. An increase in the value of CSI did not increase the risk of ALS: OR 0.81 (95% CI 0.57-1.11) p-value 0.2. Current smoking increased the risk of ALS, OR 3.62 (95% CI 1.02-13.9) p-value=0.05, a Bonferroni correction shows that this is likely a false positive result because of multiple testing. [Insert table 3 here]

[Insert figure 1 below]

**Discussion**

We found a weak association between current smoking and risk of ALS using traditional epidemiology methods to explore association. We report an uncorrected p-value of 0.05, and several models tested for fit, suggesting that this is in fact a false positive result. We also found that using CSI to measure lifetime smoking exposure resulted in a better fitting model for our data than using cigarette pack years, but we found no evidence of a dose-dependent response of ALS risk to smoking.

Our results are similar to those from a study conducted in the Netherlands which found current smoking to be associated with ALS in an incident cohort but no strong dose-dependent relationship (9). The strength of association between smoking and ALS was reported as weak in a meta-analysis of case-control and cohort studies, with a higher effect in women (7). This weakness may be due to the reliance on prevalent and clinic cohorts which would under-represent smokers because their survival is shorter (9).

A pooled analysis of prospective studies found that there was an increased risk of ALS in former and current smokers (13). Two large prospective cohort studiesincluded in the pooled analysis were originally set up as prospective studies into environmental exposures and cancer risk (11, 14). People with ALS were identified from death certificates, which may over-represent people who smoke as their survival is shorter.

The CSI is more useful than cigarette pack years to investigate dose-dependency, as it formally considers the decreased risk of disease after smoking cessation. The CSI had a bimodal distribution of smoking exposure in both cases and controls, corresponding to smoking at time of survey. The mean CSI of current smokers is slightly higher in cases than controls and so dose-dependency in current smokers should be investigated further.

Median age of smoking initiation was around the late teens in both groups, and it has been reported that frontotemporal dementia, a behavioural change that occurs in some people with ALS is not associated with smoking behaviours, so association is unlikely to reflect reverse causality (24).

The strengths of this study are that we have detailed environmental data on incident cases of ALS and controls. A limitation is the sample size which means it is only powered to detect relatively large effect sizes with odds ratios of the order of 1.8 or higher. Retrospective case-control studies generally suffer from recall bias. This study may suffer the effect of two opposing sample biases: people in an environmental study of lifestyle may be more likely to smoke heavily, and some people in this ALS study ~~are~~ attended specialist clinics so may be less likely tosmoke. Additionally, we do not know how many controls who were contacted declined to participate, so the control population may be biased. There were no current smokers in the controls recruited in London, although a subgroup analysis in the other two areas show that odds ratios for current smoking are consistent between the remaining areas.

We found that people with ALS were less likely to drink alcohol, but our survey responses so not support a protective relationship as ALS was cited as the reason for not drinking in most cases. Despite controlling for drinking and educational status, it is not possible to completely rule out the effects of confounding.

In this study of smoking and ALS, we do not find strong evidence to support smoking as a risk factor, even using lifetime smoking exposure as measured by the CSI.

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**Disclosure of interest**

None of the authors had competing interests to declare.

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|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Demographic/behavioural measure** | | **Case**  **(n=202)** | **Control**  **(n=200)** | **p-value (test)** |
| Gender ratio, Female:Male % (n) | | 41:59 (85:117) | 44:56 (88:112) | 0.77 (Chi squared test) |
| Educational attainment % (n) | Primary school | 1.5 (3) | 1 (2) | 0.0041 (Fisher’s exact test) |
| Secondary school | 38.1 (77) | 30.5 (61) |
| College | 31.2 (63) | 23.5 (47) |
| Technical school | 8.4 (17) | 12 (24) |
| University | 14.4 (29) | 29 (58) |
| Other | 5.5 (12) | 3.5 (7) |
| Missing | 0.5 (1) | 0.5 (1) | Not analysed |
| Mean age at survey (standard deviation) | | 63.1 (10.53) | 64.5 (10.52) | 0.12 (*t*-test) |
| Alcohol use % (n) | Alcohol status  Never : Ever | 8:62 (17:184) | 12:88  (24:176) | 0.32 (Fisher’s exact test) |
| Site of onset % (n) | Bulbar | 21.7 (44) | n/a |  |
| Spinal | 73.3 (148) | n/a |  |
| Not known/recorded | 5 (10) | n/a |  |
| Mean age at onset (SD) | | 60.7 (10.6) | n/a |  |
| Median months onset – diagnosis (IQR) | | 12 (13) | n/a |  |
| Median months onset – survey (IQR) | | 28.1 (21.5) | n/a |  |

Table 1: Unadjusted comparisons of demographics and behaviour in ALS cases and controls. The three centres are tertiary referral centres with about a third of the patients diagnosed at the centre, and the remainder diagnosed elsewhere first. SD = standard deviation, IQR = interquartile range n/a =Not applicable.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Smoking measure** | | **Case** | **Control** | **p-value** |
| Smoking behaviour % (n) | Smoking initiation  (ever smokers) | 47  (94) | 53  (105) | 0.27 |
| Smoking status  Never : Former : Current | 47:44:9 (94:90:18) | 53:44:3 (105:88:7) | 0.065 |
| Median age smoking initiation (n) | | 16 +- 3 (108) | 16 +- 3.5(94) | 0.94 |
| Median cigarettes per day (n) | | 17 +- 10 | 15 +- 10 | 0.88 |
| Median duration smoking | | 23.5 +- 25.6 | 23 +- 24 | 0.58 |
| Median cigarette pack years | | 20+-27.27 | 16+-28.4 | 0.7 |
| Median comprehensive smoking index values | | 0.031 +- 1.85 | 0.0053 +- 1.36 | 0.33 |

Table 2: Smoking variables and crude comparisons. Chi squared tests were used for categorical variables and Mann-Whitney U for continuous variables as all were non-normally distributed. 6 people were missing duration information, 4 missing smoking intensity. Records with missing data were excluded from analysis.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variable** | | **Odds ratio** | **Lower CI** | **Upper CI** | **P value** |
| Smoking status | Current smoker | 3.62 | 1.02 | 13.8 | 0.05 |
| Former smoker | 1.08 | 0.67 | 1.74 | 0.74 |
| Comprehensive smoking index | | 0.81 | 0.58 | 1.11 | 0.2 |
| Age | | 0.98 | 0.96 | 1 | 0.07 |
| Ever drinker | | 1.33 | 0.65 | 2.75 | 0.43 |
| Male | | 1.05 | 0.68 | 1.63 | 0.83 |
| Education level | Primary School | 2 | 0.05 | 78.4 | 0.68 |
| Secondary School | 1.27 | 0.05 | 33.2 | 0.87 |
| Technical School | 0.73 | 0.03 | 19.4 | 0.83 |
| College | 1.32 | 0.05 | 34.4 | 0.85 |
| University | 0.44 | 0.02 | 11.7 | 0.58 |
| Other | 1.6 | 0.06 | 45.7 | 0.75 |

**Table 3: Best fitting logistic regression model for smoking and risk of ALS.**

Figure 1: a) density plot of CSI value by case control status b) box plot of CSI value by smoking status at time of survey, points coloured by case control status. Both graphs are in ever smokers only.