ABSTRACT

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Risk Factors for Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive debilitating disease of the upper and lower motor neurons. Median survival of ALS patients is consistently estimated at between 2-3 years from symptom onset, with some evidence that survival is increasing due to improved care. There are few well-established risk factors for ALS, and there is conflicting evidence regarding the trends in ALS incidence and mortality over the past several decades.

In Chapter I we investigate the trends in ALS incidence and mortality in Denmark between 1970 and 2009. We employed age-period-cohort models to model both the incidence and mortality rates of ALS over time for the first time. We found a significant rise in ALS incidence and mortality over several decades, and we observed evidence for a birth cohort component to the rise in ALS, which is consistent with an environmental cause of ALS.

In Chapter II we investigate the role of physical trauma – both head and other – in the development of ALS. We employed the Danish registries and linked health data from the hospital system to prior diagnoses for physical trauma. We found a borderline significant association between physical trauma and ALS, which grew stronger upon restricting to physical traumas before the age of 55.

Chapter III concerns the risk of ALS in those employed by the military in Denmark. We linked occupational records from the Danish Pension Fund to health records of the hospital
system. We found a significantly elevated rate of ALS among those who had been previously employed by the military, with the highest rates in the decade immediately following cessation of employment.

These analyses strengthen the knowledge base for the epidemiology of ALS, and suggest future avenues of research to further understand the etiology of the disease.
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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive debilitating disease of the upper and lower motor neurons. Median survival of ALS patients is consistently estimated at between 2-3 years from symptom onset, with some evidence that survival is increasing due to improved care.\textsuperscript{1} Mortality is typically due to respiratory failure caused by the progressively paralytic nature of the disease. Prognostic factors associated with worse survival include bulbar onset, in which ALS presents with speech or swallowing difficulty, and mixed upper and lower motor neuron onset.\textsuperscript{2} Upper motor neuron involvement typically presents as muscle spasticity and hyperreflexia, whereas lower motor neuron involvement typically involves muscle atrophy and cramping. ALS occurs in Western countries at rates between 2-3 individuals per 100,000 per year.\textsuperscript{3} Incidence generally peaks around age 65, with a decreasing incidence in later ages; whether this is due to a true decline or because the disease is obscured by increasing comorbidities is still unclear.\textsuperscript{4} Men tend to experience ALS at higher rates than women.\textsuperscript{3}

About 5-10\% of patients – so called “familial” ALS cases – can be identified as relatives of other ALS cases, with the remaining 90-95\% of “sporadic” cases having no known family members. In recent decades, considerable research has been performed into the genetic risk factors for ALS, and the latest genome-wide association studies place the overall heritability of ALS near 20\%.\textsuperscript{4} The most current estimates suggest that roughly 70\% of familial ALS can be explained by known genetic mutations, whereas only 10\% of sporadic ALS can be thus explained. For both familial and sporadic ALS, the largest known contributor is a hexanucleotide repeat expansion in the gene \textit{C9ORF72}, which was discovered only in 2011.\textsuperscript{5} Other major contributors include, but are not limited to, missense mutations in the superoxide dismutase-1 gene, and mutations in the TAR DNA-binding protein.\textsuperscript{5} No consistent biochemical similarities
have been established across the range of implicated genes, but many involve breakdowns in oxide and RNA metabolism.

Very few non-genetic risk factors have been definitively linked to ALS. Smoking is associated with ALS in many studies, but a comprehensive meta-analysis found a weak overall association which, after stratification, was only apparent in women.\textsuperscript{6} Other potential risk factors include insecticides\textsuperscript{7,8}, aerosolized lead\textsuperscript{7,9}, high testosterone\textsuperscript{10}, traumatic injury\textsuperscript{11–13}, viral infections\textsuperscript{14,15}, intense physical activity\textsuperscript{16,17}, and organic solvents and formaldehyde.\textsuperscript{18,19} Studies of these risk factors, however, are often limited by the rarity of the disease. Case-control studies are often performed using convenience samples of cases and either hospital-based or case-referred controls. Moreover, the exposures of interest often require self-report spanning many years into the past. For these reasons, the quality of evidence for many of the above-mentioned putative risk factors has been deemed to be low.\textsuperscript{20}

In \textit{Chapter I} we investigate the trends in ALS incidence and mortality in Denmark between 1970 and 2009. It has been an open question whether the incidence of ALS is rising and, if it is, whether the rise is attributable to improved detection and diagnosis or a true underlying increase. By employing age-period-cohort models on both the incidence and mortality rates of ALS over time for the first time, we are able to gain a better perspective on the changing incidence of ALS over time.

In \textit{Chapter II} we investigate the role of physical trauma – both head and other – in the development of ALS. Studies have implicated both head\textsuperscript{17} and non-head\textsuperscript{21} trauma in the development of ALS, but have been limited by the study design issues mentioned above. We employ the Danish registries to capture all newly diagnosed ALS cases in a well-described
population, and make use of the Danish National Patient Registry, which includes all hospital discharge diagnoses from 1977.

**Chapter III** concerns the risk of ALS in those employed by the military in Denmark. Military service has been implicated in ALS since the first Gulf War$^{22}$, and we employ the Danish Pension Fund to obtain an objective measure of whether an individual was employed by the military in Denmark.
REFERENCES


CHAPTER I

AGE-PERIOD-COHORT ANALYSIS OF TRENDS IN AMYOTROPHIC LATERAL SCLEROSIS IN DENMARK, 1970–2009

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Abstract

Amyotrophic lateral sclerosis (ALS) is a disease of the motor neuron with poorly understood etiology. Recent studies have suggested that incidence and mortality from ALS is increasing, but it is unclear if this is due to changing exposures or improvements in diagnosis. We investigated trends in ALS incidence (hospitalization) from 1982 to 2009 and mortality from 1970 to 2009 in Denmark using age-period-cohort models. Among those 45 or older, a total of 4,265 deaths (Incidence Rate = 5.35 per 100,000 person-years) and 3,228 incident diagnoses (Incidence Rate = 5.55 per 100,000 person-years) were recorded. Age-adjusted mortality rates increased by 3.0% annually between 1970 and 2009, and 2.1% annually post-1982. Age-period-cohort analyses suggested that the full age-period-cohort model provided the best fit to the mortality data (p<0.001), although restricting to post-1982 suggested that the age-cohort model provided the best fit. Age-adjusted incidence rates increased by 1.6% annually post-1982 (p<0.001), which was best explained by the age-period model, with borderline significant cohort effects (p=0.08). A consistent finding regardless of parameterization or data subset appeared to be an increase in ALS incidence and mortality with later birth cohorts up to at least 1910.

Keywords: ALS, brain disorders, neurodegenerative, age-period-cohort, neuroepidemiology

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Abbreviations: amyotrophic lateral sclerosis (ALS); age-period-cohort (APC)
Introduction

ALS is a rare neurodegenerative disorder of unknown etiology. Although many studies have documented an apparent increase in incidence and mortality rates over time (1–5), it is unclear whether this is due to improved case ascertainment or to a genuine increase in incidence (6). Given the lack of known risk factors apart from age and possibly male sex (7), the latter hypothesis is important in that it may signal the presence of environmental and/or occupational risk factors that have changed over time.

Age-period-cohort (APC) techniques are a tool to examine rates of an outcome over time and, when changes are observed, distinguish between changes that result from birth cohort effects and period effects in the presence of a known age dependence (6,8,9). Birth cohort effects are those that impact entire birth cohorts and change their risk for some outcome over their entire life-course, relative to surrounding birth cohorts. Period effects are generally those that impact an entire population at a given moment in time, changing, either transiently or permanently, the risk of some outcome across all age groups, for example newly introduced air or water pollutants (10). We know of only two studies that have applied APC analyses to ALS population data (4,5). An analysis in France from 1968-2007 of 37,624 deaths showed an increase in mortality that was better explained by a birth cohort effect than either a period or period-cohort effect (5). An analysis in Switzerland from 1942-2008 of 5,027 deaths concluded that the increase was best explained by age-specific period effects, with steep increases limited to 1981 onwards, although they could not rule out cohort effects (4). No study has analyzed both incidence and mortality in the same population over the same years.
In order to address the apparent rise in ALS rates over time, we analyzed incidence and mortality data in Denmark starting in 1970 (mortality) and 1982 (incidence) through 2009 to determine whether or not a birth cohort effect is a likely explanation for the apparent increase in ALS incidence and mortality.

**Methods**

*Data Source*

We obtained mortality records from the Danish Cause of Death Registry, which has been kept electronically since 1970 (11). Mortality in Denmark was coded using International Classification of Diseases revision eight (ICD-8) prior to 1994, after which ICD-10 was used. Cases of ALS were defined as individuals with underlying or contributing causes of death with ICD-8 code 348.0 or ICD-10 code G12.2. The inclusion of contributing causes is generally considered the best practice, particularly for capturing ALS in the elderly (12).

We obtained data on hospitalizations from the Danish National Hospital Registry, which has collected nationwide data on all somatic hospital admissions since January 1, 1977 (13). Incident cases were defined as first inpatient discharge diagnoses with ICD-8 or ICD-10 codes as above. In order to avoid prevalent cases, we only included patient from 1982 and later. Date of first inpatient discharge was considered the case date. Date of birth was obtained from the Central Person Register, which is then linked to hospital and death registries through a personal identification number (14). We excluded all deaths (n=123) and diagnoses (n=204) of ALS in men and women less than 45 years of age. Age- and sex-specific population denominators in one-year, one-age bins were obtained from Statistics Denmark (14).
Statistical Analysis

We employed the APC method described by Carstensen (15). Briefly, to solve the identifiability problem inherent in attempting to simultaneously model age, period, and cohort, which are linearly dependent (age = period – cohort), we fix two levels and one slope among the three effects. We place no constraints on age, and constrain the cohort effect to be relative to 1920, and constrain the period effect to be relative to 1990 and be zero on average with zero slope. In this parameterization, age effects are interpretable as longitudinal rates within the reference cohort 1920 over time. Cohort effects are interpretable as the relative rate from the reference cohort (1920), in the reference period (1990). Period effects are deviations from that predicted by the age-cohort combination; this allows us to test for deviations from linear period effects over time. The estimated effects in this parameterization are dependent on the constraints used to identify them, and thus must be interpreted with caution (8). However, testing between models for goodness-of-fit is not constraint-dependent, because while the linear dependence of age, period and cohort results in non-unique effect estimates (i.e. the estimated model coefficients), the predicted values from each model are unique, and thus tests for goodness-of-fit can be performed without additional assumptions. All models were also assessed visually to check for differences in estimated effects between models.

We tabulated 1-year rates for each year of the study. In all modeling we employed natural cubic splines with 6-10 knots for each effect spaced equally at quantiles. All analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria). APC analyses were implemented using the EPI package (15).

Results

Mortality
Between 1970 and 2009 there were 4,265 deaths attributed to ALS among people older than 45 years of age for an overall mortality rate of 5.35 ALS deaths per 100,000 person-years. Age-specific male and female mortality rates for the entire study period are shown in Figure 1.1. Mortality rates peaked between 70 and 80 for both men and women and in both we observed a drop-off of rates in later years.

Over the course of the study there was an average 3.0% increase in mortality per year (age-adjusted linear period model; p<0.001). Restricted to post-1982, the linear trend was a 2.1% increase (p<0.001).

Mortality rates tabulated by age and year of death (period) are shown in Figure 1.2a. We stratified crudely for display purposes; in all APC analyses the three factors are treated continuously with splines. There was an increase in mortality rates across all age groups fifty years and above, most dramatically in those above seventy. Figure 1.2b shows mortality rates within age groups by birth cohort; older age cohorts show a greater increase in mortality rates as birth year progresses. Under the null hypothesis of neither a period nor a cohort effect, we expect both plots to exhibit parallel lines (on the log scale). That both plots show deviations from parallel indicates that neither age-period nor age-cohort models are sufficient to explain the increase.
Figure 1.1. Age-specific amyotrophic lateral sclerosis mortality in Denmark, 1970-2009.
Figure 1.2. (a) Amyotrophic lateral sclerosis (ALS) mortality rate in Denmark, 1970-2009, stratified by age at time of death. (b) ALS mortality rate by birth cohort, stratified by age at time of death. Lines correspond to each age group (solid: 40-50; short dash: 50-60; dotted: 60-70; dash-dot: 70-80; long dash: 80+).
Table 1.1 shows the results from the fit of the APC model for mortality, with age, period, and cohort year modeled continuously with splines. Compared to either the age-cohort or the age-period models, the full APC model provides a significantly better fit to the data. Figure 1.3 shows the period and cohort effects estimated from the full APC model. The period effect shown here is constrained to be zero on average with zero slope for identifiability, but there is a clear deviation from linearity in mortality from 1975-1980, when an increase occurs. The cohort effect shows a steadily increasing rate, with a possibly slightly faster increase for those born from 1930-1935. Results were unchanged when stratified by sex. When we constrained the cohort effect, rather than the period effect, to be zero on average with zero slope, the overall increase over the birth cohorts was, as expected, transferred to the period effect, but the increases in 1975-1980 (period) and prior to 1910 as well as 1930-1935 (birth cohort) remained (Web Figure 1).
Table 1.1. Modeling results from APC model for ALS mortality in Denmark, 1970-2009.\(^1\)

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\(^1\)Models are ordered so that adjacent rows provide tests between models, culminating in the age-period-cohort model. Change in residual degrees of freedom (DF) and deviance are used to perform a chi-squared test between adjacent models, where the fuller model is accepted if the test is significant.

Abbreviations: APC – age-period-cohort; ALS – amyotrophic lateral sclerosis; DF – degrees of freedom
Figure 1.3. Age-period-cohort model of all amyotrophic lateral sclerosis mortality in Denmark, 1970-2009, with average period effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
When we limited mortality data to post-1982 (for comparability to the incidence data), the age-cohort model formally provided the best fit to the data, although the full APC model was only marginally non-significant (p=0.10 comparing the age-cohort to the full model). Plots of the effects from this full APC model (Web Figures 2 and 3) are similar to those from the complete mortality data, (Figures 1.3 and Web Figure 1) for the years they share. Because Denmark switched from ICD-8 to ICD-10 in 1994, we also restricted mortality data to post-1994, and again the age-cohort model was a better fit than the full APC model (p=0.78 comparing the age-cohort to the full model). In all of the treatments of the mortality data, the increase in ALS with later cohorts prior to at least 1910 is consistent.

We stratified results by age to address the possibility that improved diagnosis in the elderly over time manifests as a cohort effect (an age-period interaction). When individuals 80 and older were excluded, results were largely unchanged. The full age-period-cohort model was still strongly preferred (p=<0.001). When individuals over the age of 65 were excluded, the period effect failed to reach significance when added to the age-cohort model (p=0.18).

**Incidence**

Between 1982 and 2009 there were 3,228 newly diagnosed ALS cases recorded among people older than 45 years of age, for an overall incidence rate of 5.55 cases per 100,000 person-years. Figure 1.4 displays age-period and age-cohort plots for incidence rates. Unlike for mortality, age-specific incidence rates were approximately linear over the period 1982-2009, with some evidence of a slight increase across all ages. Age-specific incidence rates by birth cohort also exhibit the pattern we expect from an age-cohort model, with the possible exception
of individuals at least 80 years old. The age-adjusted linear increase in incidence rates over the study period was 1.6% per year (p<0.001).
Figure 4. (a) Amyotrophic lateral sclerosis (ALS) incidence rate in Denmark, 1982-2009, stratified by age at time of diagnosis (discharge). (b) ALS incidence rate by birth cohort, stratified by age at time of diagnosis (discharge).
The APC modeling results (with age, period, and cohort year modeled continuously with splines) formally indicated that the age-period model provided the best fit to the data, although the full APC model was only marginally non-significant (p=0.08; Table 1.2). Plots from full APC models for incidence showed a slight period increase between 1992 and 1996, and a cohort effect of increasing incidence over birth cohorts prior to 1920 (Figure 1.5), mirroring the mortality data, in particular the post-1982 mortality data. As in the mortality data, reparameterizing the model to constrain the cohort effect, rather than the period effect, to be zero on average with zero slope did not materially change these results (Web Figure 4), with some of the overall increase being transferred to the period effect. Of note, though, even in this reparameterization, the increase with increasing birth cohorts before 1910 was still seen. Results were similar when stratified by sex, although there was evidence of a stronger birth cohort effect among women, particularly among later birth cohorts (post-1940). Because the cohort effect was borderline significant, we also considered the alternative age-period-interaction model (Web Table 1).

**Sex ratio**

For both incidence and mortality, male and female rates converged over time up to 1995-2000 and began to diverge thereafter. The relative mortality rate ratio in women relative to men rose from 0.42 to 0.88 from 1970-1975 to 1995-2000, and decreased to 0.71 by 2005-2010. The relative incidence rate ratio for women relative to men rose from 0.59 to 0.91 from 1982-1985 to 1995-2000, and decreased to 0.80 by 2005-2010 (Figure 1.6).
Table 1.2. Modeling results from APC model for ALS incidence in Denmark, 1982-2009.\(^1\)

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\(^1\)Models are ordered so that adjacent rows provide tests between models. Change in residual degrees of freedom (DF) and deviance are used to perform a chi-squared test between adjacent models, where the fuller model is accepted if the test is significant.

Abbreviations: APC – age-period-cohort; ALS – amyotrophic lateral sclerosis; DF – degrees of freedom
1.5. Age-period-cohort model of amyotrophic lateral sclerosis incidence in Denmark, 1982-2009, with average period effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
Figure 1.6. Relative amyotrophic lateral sclerosis mortality and incidence rates in women vs. men in Denmark, 1970-2009.
Discussion

Our results suggest that the increase in both mortality and incidence of ALS in Denmark has a birth cohort component. If this holds, it suggests the existence of a behavioral or environmental factor driving ALS incidence that occurs in particular age groups at particular times, whether it be early in life (a true “birth” cohort effect)—e.g. in utero or childhood exposure—or as they enter the workforce, or later in life. These results also suggest that period effects – those that affect the entire population at a given calendar period – are less important in explaining the trends in ALS mortality.

Several studies have suggested a possible role of behavioral and environmental factors in ALS (16), but the possibility that exposure to such factors early in life has not been extensively explored. One report found an increased risk of ALS among those who played varsity sports when younger (17), although others have not found associations with early sports activity (18,19). A recent study reported that higher testosterone levels in utero—as assessed by examining the difference in lengths of the 2nd and 4th fingers—were related to subsequent increased risk of ALS (20).

One prior study of ALS in France found largely similar results: a steep increase in motor-neuron disease mortality between birth years 1883-1923, with a subsequent plateau. However, they found that age-cohort models adequately explained the change in mortality (5). In contrast, we found that mortality rates were best explained by both cohort and period effects (in addition to age). While we observed the early cohort increase from 1880-1920, we also observed a period increase in ALS mortality prior to 1982. This latter finding replicates results from the United States of an increase in mortality prior to 1983 with a subsequent plateau, although those results were not simultaneously controlled for year of birth (1). A prior study in Switzerland with
records beginning in 1942 came to the conclusion that age-specific period effects were a sufficient explanation for the change in mortality rates, which may suggest that improved diagnosis before the 1970’s overwhelmed any birth cohort effects that may have occurred later, while from the 1970’s onward improvements in diagnoses were less important relative to birth cohort effects (4).

Prior studies have observed that rates in men and women have been converging over time (5,16,17). We found a similar pattern in Denmark in mortality and report for the first time a similar trend in incidence. One possible explanation for this is that environmental and occupational factors, as well as smoking, have become increasingly balanced between sexes, and there is evidence suggestive of a causative role of such exposures (7,21,22). Alternatively, the convergence could be explained by improved diagnosis among women relative to men, which is unlikely because of free health care for all in Denmark independent of workforce involvement. However, as for our main findings, any such improved diagnosis would have to affect incidence and mortality equally since we found similar results for the sex ratio of ALS in incidence and mortality data. Thus, for example, more improvement in identification of ALS on death certificates for women than men could not explain our findings. Improved diagnosis of ALS among women, if that also led to a similar improved identification on death certificates, could possibly explain our findings, but this would have to apply to diagnosis at any age since results excluding those over 65 or 80 years old were similar.

The major strengths of this study are its size and time-span, and the availability of both incidence and mortality data. In addition, the use of APC techniques allows us to test for the presence of birth cohort effects while simultaneously controlling for shifts in period-specific mortality and incidence. While a strength of using National registries is the size and
completeness it provides, problems can arise if diagnostic accuracy is low. The general validity of the Danish Hospital Register is considered to be high (23), and both diagnostic sensitivity and specificity are generally reported to be quite high (e.g. 84% or >90%) using hospital discharge codes for ALS, although positive predictive value can be slightly lower, with positive predictive value generally being better for mortality data than hospital discharge data (24–26). In a previous study, however, medical records were obtained for 15 incident ALS cases identified by ICD code in the Danish Hospital Register and all 15 cases were confirmed as ALS (27). A related limitation is the switch in 1994 from ICD-8, which was unique to ALS, to ICD-10, which includes other motor neuron diseases. However, our analysis of mortality limited to post-1994 deaths showed no major difference in results.

Limitations of our study include the requirement for arbitrary constraints to achieve identifiability of the effects of age, period, and cohort. There are no solutions to the problem of identifiability that are entirely free of the constraint problem. However, by examining the range of possible constraints, we can estimate the range of plausible effect estimates. Our exploration of alternate constraints did not change our qualitative findings of a large pre-1920 (birth cohort) increase in both mortality and incidence. This pre-1920 birth cohort effect did not account for all of the rise in ALS incidence and mortality seen, but how much of the remaining rise can be attributed to a cohort or period effect is hard to determine given the results of the two parameterizations. The alternate parameterizations also did not change the findings of an increase in mortality among those born 1930-1935 and those living around 1980.

Although the cohort effect was borderline insignificant for incidence data, this could be explained by the difference in calendar years for incidence versus mortality. The twelve fewer years of incidence data allow for less power in detecting effects, particularly those effects that
manifest in older cohorts – precisely where the most consistent, regardless of parameterization choice, increases with birth cohort were seen. Importantly, plots of all mortality and mortality restricted to the years of the incidence data (post-1982) were similar, and plots of incidence and mortality were similar. For these reasons, we emphasize the full APC model for the incidence data, although a plausible alternative is the age-period-interaction model, results of which are shown in Web Table 1. In that case, period effects would be represented by the age-stratified rates as in Figure 4a.

A further limitation inherent in APC analyses is that we cannot directly address the hypothesis that ascertainment improved differentially with respect to age – in particular, that diagnosis improved in the elderly over the study period. Such an effect would appear as a cohort effect, while in reality it would be best considered an age-period interaction (with an effect in particular ages at particular periods). For example, an increase in rates among those at least 65 years old in 2000 could be explained by either a cohort effect (the introduction of an exposure in 1935) or an age-specific period effect (improved case ascertainment among the elderly beginning in 2000). The similarity between our findings for both incidence and mortality rates, however, suggest that any such changes would have to affect both incidence and mortality and could not be, for example, better recognition of ALS on death certificates among the elderly. Nor could they affect men and women differently as we generally found similar patterns for men and women.

In summary, this large national study provides evidence for a substantial increase in ALS incidence and mortality in Denmark in succeeding birth cohorts from 1880 to 1920 with a subsequent plateauing, and suggests a convergence in risk for men and women by advanced calendar time. These results support an environmental cause of ALS that became more common
in the 20th century, in a way that impacted successive birth cohorts as whole units, particularly those born pre-1920. Given the paucity of known risk factors, these findings may help narrow future research into environmental agents based on their historical use patterns.
References


Web Appendix

**Web Table 1.** Test for age-period-interaction model vs. the simpler age-period model for ALS incidence in Denmark, 1982-2009.1

<table>
<thead>
<tr>
<th></th>
<th>Residual DF</th>
<th>Residual Deviance</th>
<th>Change in DF</th>
<th>Change in Deviance</th>
<th>Pr(&gt;Chi)</th>
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<td>Age-Period</td>
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<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Age-Period-Interaction</td>
<td>2512</td>
<td>2792</td>
<td>49</td>
<td>78</td>
<td>0.005</td>
</tr>
</tbody>
</table>

1Models are ordered so that adjacent rows provide tests between models. Change in residual degrees of freedom (DF) and deviance are used to perform a chi-squared test between adjacent models, where the fuller model is accepted if the test is significant.

Abbreviations: ALS – amyotrophic lateral sclerosis; DF – degrees of freedom
Web Figure 1. APC model of all ALS mortality in Denmark, 1970-2009, with average cohort effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
Web Figure 2. APC model of post-1982 ALS mortality in Denmark, 1970-2009, with average period effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
**Web Figure 3.** APC model of post-1982 ALS mortality in Denmark, 1970-2009, with average cohort effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
Web Figure 4. APC model of ALS incidence in Denmark, 1982-2009, with average cohort effect constrained to be zero. (a) Estimated period effects relative to 1990. (b) Estimated birth cohort effects relative to 1920.
CHAPTER II

PHYSICAL TRAUMA AND ALS: A POPULATION-BASED STUDY IN THE DANISH NATIONAL REGISTRIES

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Abstract

Prior studies have suggested that physical trauma may be associated with the development of amyotrophic lateral sclerosis (ALS). We conducted a population-based, individually-matched case-control study in Denmark to assess whether hospitalization for trauma is associated with an increased risk of developing amyotrophic lateral sclerosis. There were 3,650 incident cases of amyotrophic lateral sclerosis in the Danish National Patient Register from 1982 to 2009. Each case was matched to 100 age- and sex-matched population controls alive on the date of the case diagnosis via risk set sampling, and odds ratios (OR) and confidence intervals (CI) were calculated via conditional logistic regression. History of trauma diagnosis was also obtained from the Danish Patient Register. When traumas in the five years prior to the index date were excluded, there was a borderline association between any trauma and ALS (OR =1.09; 95% CI 0.99, 1.19). A first trauma before age 55 was associated with ALS (OR=1.22; 95% CI 1.08, 1.37), while first traumas at older ages were not (OR=0.97; 95% CI 0.85, 1.10). Our data suggest that physical trauma at earlier ages is associated with ALS risk. Age at first trauma could help explain discrepancies in results of past studies of trauma and ALS.

Keywords: ALS, Amyotrophic lateral sclerosis, Neurology, Physical trauma

Abbreviations:

ALS – amyotrophic lateral sclerosis
OR – odds ratio
CI – confidence interval
ICD – International Classification of Diseases
SES – socioeconomic status
Introduction

Little is known about the causes of amyotrophic lateral sclerosis (ALS). Clinical observations and some case-control studies have indicated that head trauma may be a risk factor for ALS (1,2). Trauma to the head is known to disrupt the blood-brain barrier (3), which is selectively impermeable to many solutes, including some toxins. It has been hypothesized that deterioration of the barrier may play a role in ALS pathogenesis (4). Head trauma has also been implicated in the development of other neurodegenerative diseases, including Alzheimer’s disease and Parkinson’s disease, both of which share some pathologic and epidemiologic characteristics with ALS (5,6). Head trauma is known to induce glutamate excitotoxicity, be associated with mitochondrial dysfunction, neuroinflammation, and cause endoplasmic reticulum stress, all of which have been implicated in ALS pathogenesis (7–9).

Early case-control studies consistently found a significant association between history of head trauma and risk of ALS. A 2007 meta-analysis of nine studies reported an odds ratio of 1.7 (95% CI: 1.3, 2.2) (2). Many of these early studies, however, were characterized by self-reported trauma history, creating the potential for recall bias, and by a lack of proper exposure lagging. In order to reduce the problem of reverse causation – in which incipient ALS causes trauma – more recent studies generally exclude traumatic events in either the three or five years prior to the index date (10–12). A recent large prospective study based on objective assessment of head trauma found no association between hospitalization for head trauma and ALS when traumas in the 3 years before ALS were excluded (11). Thus, it is unclear whether or not early signs of a link between head trauma and ALS will persist when these timing aspects are considered, and whether or not particular patterns of trauma – for example location, severity, frequency, or timing of traumatic injuries – will be established as risk factors for ALS. There is also a limited
and conflicting literature on the role of other physical traumas in the development of ALS, but there is little large scale cohort data on this question (13,14).

The objective of this study was to assess the risk of ALS following physical trauma (head and other) using national registry data in the Danish population. We hypothesized that ALS patients would have higher rates of physical trauma, particularly head trauma, and that the number and severity of traumatic events would be correlated with ALS risk.
Methods

Case Ascertainment

The Danish National Patient Register contains data on hospitalizations in the inpatient setting (since 1977) and in the outpatient setting (since 1995). The outpatient setting captures visits to outpatient clinics or emergency rooms. We identified hospitalizations with a discharge diagnosis of ALS from the registry (International Classification of Diseases, ICD-8 348.0 or ICD-10 G12.2). The discharge diagnosis includes both the “action diagnosis” and the “cause of treatment.” An example would be treatment for kidney insufficiency (“action diagnosis”), which is caused by diabetes (“cause of treatment”).

ALS diagnoses were therefore inpatient only prior to 1995, and both inpatient and outpatient thereafter. Overall, 1,567 cases (42.9%) were first identified from outpatient records, with the remainder from inpatient records. After 1/1/1995, when outpatient records were available, this proportion increased to 61.6%. Overall, 1,875 (51.4%) of cases had both inpatient and outpatient diagnoses for ALS; in these individuals the outpatient record preceded the inpatient record in 1,119 (59.7%) cases.

ICD-8 codes were used in Denmark through 1994, with ICD-10 thereafter. In a validation substudy of 173 ALS cases identified this way, we obtained medical records and confirmed at least a clinically suspected ALS diagnosis in 160 (92.5%), and we observed no difference between ICD-8 and ICD-10 codes (15). The National Patient Register collects data on all hospital admissions nationwide, beginning on January 1, 1977 (16). We limited our case definition to first diagnoses on or after January 1, 1982, a five-year washout period to reduce the inclusion of prevalent cases. For the present study, cases were collected through December 31,
2009, and the index date was the first recorded hospitalization with ALS recorded as the primary discharge diagnosis.

Controls

We matched 100 controls to each case on sex and age, in 1-year windows, who were alive and free of an ALS diagnosis on the index date (risk-set sampling). Controls were drawn from the Central Person Registry, a registry covering all residents in Denmark since 1968 (17). All inhabitants of Denmark are assigned a unique 10-digit Central Person number, which encodes information on date of birth and sex, and can be used to link between information from the Central Person Registry and several health related registries, including the Patient Register. Both cases and controls were required to be living in Denmark on the index date. Present and historical information from this register is kept if a person has died or immigrated.

Covariates

In addition to the matching variables of age, sex, and calendar date, we abstracted information on highest socioeconomic status (SES) attained, marital status and history, and residence from the Central Person Registry on the index date. SES was classified into five groups based on an individual’s and, if applicable, his or her spouse’s job titles: academics and managers (1), high salaried (2), low salaried (3), skilled workers (4) and unskilled worker (5). When both an individual and his or her spouse’s highest job title category were reported, we used the higher SES category (lowest number) of the two. If neither was reported, the individual was most likely unemployed, and we categorized these individuals’ SES as ‘Unknown’. Marriage status was categorized as married, unmarried, divorced, or widowed as of the index
date. Residence at the index date was classified into Copenhagen, Copenhagen suburbs, Aarhus/Odense, provincial town, rural areas, Greenland, and unknown.

**Exposure Assessment**

We reviewed case and control hospital records for history of trauma diagnoses, and classified them as any trauma, head trauma (intracranial injuries), or other traumas (traumas not classified as head) based on ICD-8 and ICD-10 codes (Web Appendix 1).

We defined any prior trauma for each of the two types as any history of a diagnosis, outpatient or inpatient, prior to the index date. The majority of analyses exclude the five years prior to the index date, to allow for latent ALS. For each individual we calculated the age at which they experienced their first and last recorded trauma, with the restriction that both occurred more than five years prior to the index date; we then categorized this into groups of no trauma (reference) and categories of age among those with trauma (<35 years, 35-54, 55-74, and 75+). We further categorized diagnoses into number of total diagnoses of each type, and duration of the longest held diagnosis (longest duration of hospitalization) as a measure of trauma severity. For each of the three trauma categories (any, head, and other), we also calculated the amount of time between the index date and the date of all traumas. We then categorized times into up to 1 year after ALS diagnosis, up to 1 year prior, 1-5 years prior, and 5+ years prior to ALS diagnosis. We included the 1 year before and after ALS diagnosis to demonstrate the magnitude of reverse causality, and the importance of ignoring exposures close to the diagnosis date. For all traumas, only traumas occurring >1 day apart were considered separate events. Results were unchanged when this was extended to >6 days apart.
Because inpatient and outpatient trauma diagnoses may differ in type and severity, we performed similar analyses separating inpatient and outpatient trauma diagnoses (trauma diagnosis type). All trauma histories for these analyses were restricted to post-January 1, 1995, to ensure comparability between inpatient and outpatient histories, because this is the date from which outpatient histories are available. A trauma diagnosis was classified as inpatient or outpatient, where outpatient visits were classified as visits to outpatient clinics or emergency rooms not followed by an inpatient record within 1 day; otherwise the visit was considered an inpatient trauma.

**Negative Control**

To assess whether confounding by smoking was a likely explanation for any observed association between trauma and ALS, we conducted a negative control analysis of the association between trauma and lung cancer (18). Briefly, if smoking confounds the trauma-ALS association then it would also confound the trauma-lung cancer association (Web Appendix 2). We thus conducted a case-control study of lung cancer among the ALS controls, with lung cancer controls selected from those without a lung cancer diagnosis by the matched case’s age and diagnosis date. Among controls from the original ALS study (n=365,000), we identified hospitalizations with a primary discharge diagnosis of lung cancer from the Danish National Patient Register (ICD-8: 162 or ICD-10: C34). For each case, we matched up to 4 controls who were free of a lung cancer diagnosis on the index date (risk-set sampling). Controls were matched on the same criteria as in the primary ALS study (sex, age, and calendar year).

**Statistical Analysis**
All models are conditional logistic regressions with strata defined by the 1:100 matched case-control sets in the case of ALS analysis, or 1:4 matched case-controls sets in the case of lung cancer negative control analyses. Given the risk-set sampling, odds ratios from these models estimate rate ratios. We adjusted all models for the matching factors (age, sex, and calendar year) as well as for SES, area of residence, and marital status as of the index date. We categorized the history of physical traumas by which unique combination of head and/or other traumas an individual suffered (‘Head’, ‘Other’, ‘Head & Other’). In models where head and other physical traumas are separated they are entered into models simultaneously; for example, the odds ratios for number of hospitalizations due to head and other traumas are obtained from models that include both.

Linear trends for age at first trauma were assessed with a continuous term for year of first trauma; in this model we also included an indicator for ever having had a trauma, because the linear trend variable (age at first trauma) was not defined for individuals with no trauma history. A similar approach was used to test the trend for duration of longest stay. We assessed the linear trend for number of hospitalizations by entering the number of hospitalizations as a continuous variable. We tested for effect modification by age and sex, with a cutoff of $\alpha=0.1$ to identify potentially significant modification.

All analyses were performed in SAS 9.3 (Cary, NC). Graphics were produced in R (CRAN, Vienna, Austria). This study was determined to be exempt by the Harvard School of Public Health IRB and was approved by the Danish Data Protection Agency. The analysis consisted solely of secondary analysis of existing data; therefore, informed consent was deemed unnecessary.
Results

Between January 1, 1982 and December 31, 2009 there were 3,650 diagnosed cases of ALS in Denmark. Table 2.1 shows descriptive statistics for cases and controls. Cases had a median age of 67 years. Cases and controls were similar with regard to residence and SES, with cases being slightly more likely to reside in Copenhagen and to have a higher SES. Cases were more likely to be married, and less likely to be widowers, at the time of case diagnosis or index date.

There was a strong association between unlagged trauma history and ALS (OR=1.43; 95% CI 1.33, 1.54), but associations attenuated upon exclusion of the prior five years of trauma history (OR=1.09; 95% CI 0.99, 1.19) (Table 2.2). When physical trauma was split by type, we observed an elevated rate of ALS in those who had a history of both head and other traumas prior to five years before the index date (OR=1.40; 95% CI 1.09, 1.80). When this was further adjusted for total number of traumas experienced (5-year lag), point estimates were somewhat more elevated for ‘Head & Other’ and ‘Other only’, and the odds ratio for those who experienced only a head trauma more than five years in the past (‘Head only’) was elevated (OR=1.12; 95% CI 0.54, 2.33). The high correlation between total number of traumas and type of trauma made all confidence intervals substantially wider.
Table 2.1. Descriptive statistics of ALS cases and controls, Denmark, January 1 1982 – December 31 2009.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Male sex</td>
<td>1954</td>
<td>53.5</td>
</tr>
<tr>
<td>Year of birth\textsuperscript{b}</td>
<td>1932</td>
<td>14</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>200</td>
<td>5.5</td>
</tr>
<tr>
<td>45-54</td>
<td>420</td>
<td>11.5</td>
</tr>
<tr>
<td>55-64</td>
<td>926</td>
<td>25.4</td>
</tr>
<tr>
<td>65-74</td>
<td>1251</td>
<td>34.3</td>
</tr>
<tr>
<td>74-85</td>
<td>757</td>
<td>20.7</td>
</tr>
<tr>
<td>≥85</td>
<td>96</td>
<td>2.6</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen</td>
<td>525</td>
<td>14.4</td>
</tr>
<tr>
<td>Copenhagen suburbs</td>
<td>787</td>
<td>21.6</td>
</tr>
<tr>
<td>Aarhus/Odense</td>
<td>405</td>
<td>11.1</td>
</tr>
<tr>
<td>Provincial towns</td>
<td>1350</td>
<td>37.0</td>
</tr>
<tr>
<td>Rural areas</td>
<td>525</td>
<td>14.4</td>
</tr>
<tr>
<td>Greenland</td>
<td>6</td>
<td>0.2</td>
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</tr>
<tr>
<td>1</td>
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<td>426</td>
<td>11.7</td>
</tr>
<tr>
<td>3</td>
<td>710</td>
<td>19.5</td>
</tr>
<tr>
<td>4</td>
<td>944</td>
<td>25.9</td>
</tr>
<tr>
<td>5</td>
<td>678</td>
<td>18.6</td>
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<td>Marriage Status</td>
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<tr>
<td>Unmarried</td>
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<td>Divorced</td>
<td>359</td>
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</tr>
<tr>
<td>Widower</td>
<td>795</td>
<td>21.8</td>
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</table>

Standard deviation (sd); socioeconomic status (SES)
\textsuperscript{a}N = 3,650 cases and 365,000 controls. Characteristics describe the cases and controls on the case (index) date.
\textsuperscript{b}Values are means and standard deviation
\textsuperscript{c}SES ranges from 1 (High) to 5 (Low)
Table 2.2. Adjusted\(^a\) odds ratios and 95% confidence intervals (CI) for association between any, head, and other traumas with ALS in Denmark, Jan 1 1982-Dec 31 2009.

<table>
<thead>
<tr>
<th>Type of Trauma</th>
<th>Unlagged Controls</th>
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<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>OR(^a)</td>
<td>95% CI</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
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<td>73.5</td>
<td>2459</td>
<td>67.4</td>
<td>1</td>
<td>ref</td>
<td>303131</td>
<td>83.1</td>
<td>3003</td>
<td>82.3</td>
</tr>
<tr>
<td>Any trauma</td>
<td>96780</td>
<td>26.5</td>
<td>1191</td>
<td>32.6</td>
<td>1.43</td>
<td>1.33, 1.54</td>
<td>61869</td>
<td>17.0</td>
<td>647</td>
<td>17.7</td>
</tr>
<tr>
<td>Head only</td>
<td>3133</td>
<td>0.9</td>
<td>41</td>
<td>1.1</td>
<td>1.51</td>
<td>1.11, 2.06</td>
<td>2714</td>
<td>0.7</td>
<td>22</td>
<td>0.6</td>
</tr>
<tr>
<td>Head &amp; Other</td>
<td>7507</td>
<td>2.1</td>
<td>96</td>
<td>2.6</td>
<td>1.55</td>
<td>1.26, 1.91</td>
<td>5028</td>
<td>1.4</td>
<td>65</td>
<td>1.8</td>
</tr>
<tr>
<td>Other only</td>
<td>86140</td>
<td>23.6</td>
<td>1054</td>
<td>28.9</td>
<td>1.42</td>
<td>1.31, 1.53</td>
<td>54127</td>
<td>14.8</td>
<td>560</td>
<td>15.3</td>
</tr>
</tbody>
</table>

Odds ratio (OR); Confidence interval (CI)

\(^a\)All models adjusted for matching factors (age, sex and calendar date), residence, marital status, and SES.

\(^b\)Lagged analyses exclude history of trauma within 5 years of index date. The reference group for all odds ratios is those with no history of physical trauma.
Table 2.3. Adjusted\(^a\) odds ratios and 95% confidence intervals (CI) for association between duration and number of physical traumas of each type with ALS in Denmark, Jan 1 1982-Dec 31 2009.

<table>
<thead>
<tr>
<th>Type, No., or Duration of Trauma</th>
<th>Controls</th>
<th>Cases</th>
<th>OR(^a)</th>
<th>95% CI</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>No Trauma</td>
<td>303131</td>
<td>3003</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td># of hospitalizations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>36866</td>
<td>366</td>
<td>1.03</td>
<td>0.92, 1.15</td>
</tr>
<tr>
<td>2</td>
<td>13742</td>
<td>153</td>
<td>1.16</td>
<td>0.98, 1.37</td>
</tr>
<tr>
<td>3+</td>
<td>11261</td>
<td>128</td>
<td>1.22</td>
<td>1.01, 1.46</td>
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<tr>
<td>Linear trend ((P) value)</td>
<td></td>
<td></td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Duration of longest hospitalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1 days</td>
<td>25626</td>
<td>282</td>
<td>1.13</td>
<td>1.00, 1.29</td>
</tr>
<tr>
<td>2-6 days</td>
<td>12155</td>
<td>130</td>
<td>1.12</td>
<td>0.94, 1.34</td>
</tr>
<tr>
<td>7+</td>
<td>24088</td>
<td>235</td>
<td>1.02</td>
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<tr>
<td>Linear trend ((P) value)</td>
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<td>0.28</td>
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<tr>
<td>Head trauma(^b)</td>
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<td></td>
<td></td>
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<tr>
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<td>3563</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td># of hospitalizations</td>
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<td>6706</td>
<td>77</td>
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<td>0.91, 1.45</td>
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<tr>
<td>2+</td>
<td>1036</td>
<td>10</td>
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<td>0.39</td>
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<td>Duration of longest hospitalization</td>
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<td></td>
</tr>
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</tr>
<tr>
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<td>0.75, 1.39</td>
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<tr>
<td>Linear trend ((P) value)</td>
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<td></td>
<td></td>
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<td>No Other Trauma</td>
<td>305845</td>
<td>3025</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td># of hospitalizations</td>
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<td></td>
<td></td>
</tr>
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<td>358</td>
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<td>Duration of longest hospitalization</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0-1 days</td>
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<td>0.98, 1.28</td>
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<tr>
<td>Linear trend ((P) value)</td>
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<td></td>
<td>0.21</td>
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</tbody>
</table>

Odds ratio (OR); Confidence interval (CI)

\(^a\)All models adjusted for matching factors (age, sex and calendar date), residence, marital status, and SES. All analyses exclude history of trauma within 5 years of index date. The reference group for all odds ratios is those with no history of that type (Any, Head, Other) physical trauma.

\(^b\)Models for # of hospitalizations and duration of longest hospitalization for head trauma and other trauma include both terms (i.e. # of hospitalizations for head trauma is adjusted for # of hospitalizations of other trauma, and vice versa).
When we categorized physical traumas by number of times hospitalized and duration of longest hospitalization, we observed an association with increasing number of hospitalizations for any type of physical trauma and number of non-head physical traumas, where the number of head and non-head physical traumas are modeled simultaneously. The linear test for trend was borderline significant for both number of any traumas ($P=0.049$) and other traumas ($P =0.075$) (Table 2.3). There was no evidence that increasing duration of physical trauma hospitalization was associated with increased ALS rates. There was no evidence of interaction between head and other traumas for either number of hospitalizations or duration of longest hospitalization.

We observed a decreasing association between age at first physical trauma diagnosis (5-year lagged) and ALS rate (Figure 2.1; linear trend $P =0.009$). Among those with a first trauma diagnosis (any trauma) before the age of 35, the odds ratio for ALS was 1.35 (95% CI 1.05, 1.72). A first trauma diagnosis date after the age of 55 was not associated with an increased odds of ALS (OR=0.97; 95% CI 0.85, 1.10). We observed a similar pattern when we considered age at last trauma (data not shown).
Figure 2.1. Adjusted odds ratios (reference is those without the trauma) for ALS by age of first trauma diagnosis Jan 1 1982-Dec 31 2009.
For all three physical trauma types (any physical trauma, any head trauma, and any other physical trauma), there was a strong and significant association between trauma and ALS diagnosis in the year following the index date (Figure 2.2). By five years associations with all three categorizations of trauma had reverted to the null.

When we looked separately at physical trauma in the inpatient and outpatient settings among the 2,409 cases and 240,900 controls after outpatient data became available in 1995, there was no evidence of an association between 5-year lagged inpatient trauma visits and ALS. However, any trauma in the outpatient setting was associated with ALS after excluding the five years before diagnosis (OR=1.18; 95% CI 1.04, 1.33) (Table 2.4). Those with a history of other traumas in the outpatient setting also exhibited an association with ALS (OR=1.16; 95% CI 1.02, 1.31).

Among controls, outpatient head traumas were dominated by concussions (39%) and traumatic subdural hemorrhage (32%), followed by diffuse brain injury (11%). Among cases, however, the most common outpatient head traumas were epidural hemorrhage (33%) and diffuse brain injury (27%), while concussions made up only 20% of the diagnoses in cases. ‘Other’ physical traumas were dominated by fractures and strains to the extremities, with no apparent differences between cases and controls in relative frequencies.

There was no significant modification of any of the associations by either sex or age, as dichotomized at the median age at index date of 67 years. Neither was there a substantial difference when results were split by case year before or after 1995, which is when outpatient results were included, and 1 year after the switch from ICD-8 to ICD-10. The negative control analyses did not suggest confounding of the findings by smoking (Web Table 1, Web Table 2).
Figure 2.2. Adjusted odds ratios (reference is those without the trauma) for ALS by timing of trauma relative to ALS diagnosis Jan 1 1982-Dec 31 2009.
Discussion

In this study covering all hospitalized cases of ALS in Denmark between 1982 and 2009, we observed that physical traumas occurring earlier in life were associated with an increased rate of ALS. We also found a significant association between physical traumas resulting in outpatient visits and ALS, with a strong association with ‘Head & Other’ traumas specifically, even after excluding traumas in the 5 years before ALS. This is the first time that a significant association has been observed between ALS and trauma when trauma history is obtained from objective registry data and an appropriate window of time prior to ALS diagnosis has been excluded.

Early studies of physical trauma and ALS were plagued by three issues: small size, insufficient lagging of exposure, and self-recall of trauma history (2). Two recent larger studies avoided some of these limitations, but came to opposite conclusions. Pupillo et al. employed a regional Italian ALS registry to identify cases, matched to hospital controls (10). While the study relied on self-reported trauma history, they were able to adjust for potential confounders. After exclusion of traumas within five years of ALS index date, they observed an elevated association with head and limb trauma. Peters et al. employed the Swedish National Registry system and observed no significant association between severe head trauma and ALS risk after exclusion of head traumas within three years of ALS index date (11). The Swedish registry study, however, did not consider non-head injury traumas, the age at first trauma, nor did it distinguish between outpatient and inpatient traumas. Our overall results on head trauma agree with theirs, and suggest that considering head injury in isolation may be insufficient.

Physical traumas early in life may be associated with ALS simply because of a long latency between exposure and outcome. In our data, ALS patients with younger ages at first
trauma had longer periods between that trauma and their ALS diagnosis, although there was substantial overlap in the distributions. It is also possible that earlier traumas may be of a qualitatively different type than later-life traumas, or that earlier-life traumas may be more subject to confounding by physical activity, although the evidence for physical activity as a risk factor for ALS is mixed (19,20). Alternatively, it has been hypothesized that higher testosterone is associated with ALS, and it could be that those prone to early life traumas have higher levels of testosterone (21). A potential causal mechanism underlying an association between trauma and ALS is trauma-induced inflammation, which is suspected of playing a role in other neurodegenerative disorders (22,23).

Our study is the first to consider the association with ALS of inpatient and outpatient visits for physical trauma separately. A recent study in the Danish registries of disease trajectories, not limited to trauma or ALS, found that type of visit (inpatient vs. outpatient) was as important in predicting the future trajectory of medical care as was age or sex (24). Our findings could be explained if people who show up in the outpatient setting for traumas are more likely than people who show up in the inpatient setting to have other less severe, but more common, traumas that are not captured in the Patient Register, under the assumption of a true causal relationship between trauma and ALS. An exploration among the controls of specific trauma types occurring in the outpatient and inpatient setting did not reveal strong differences.

The most notable limitation of the present study is the inability to directly control for behavioral confounders, such as tobacco smoking and physical activity. Although few risk factors have been established for ALS, smoking (25,26), physical activity (19) and pre-morbid body mass (27) have been implicated. Evidence is strongest for smoking, although these studies generally do not show a consistent dose-response relation and several studies suggest that the
association exists only among women (25,26,28,29). To attempt to address possible confounding by smoking, we used a negative control approach (18). Overall, we observed little evidence of residual confounding by smoking in these analyses. The exposures for which ALS risk appeared to be elevated – age at first trauma and outpatient traumas – exhibited the least evidence of residual confounding (Web Table 2).

Because the hospital registries were established only in 1977 (inpatient) and 1995 (outpatient), we may miss older physical traumas. The analysis of age at first trauma is particularly susceptible to this problem, as are the overall analyses if, in fact, there is a substantial latency between traumatic event and ALS risk. The registry design, however, makes it highly unlikely that this misclassification is related to ALS risk and would therefore bias results towards the null.

The strengths of the present study are its overall size, national comprehensiveness and representativeness, and objective registry-based diagnosis of ALS and physical trauma exposures. While a limitation of this method of exposure assessment is that only trauma meriting an inpatient (1977 and later) or outpatient (1995 and later) visit is captured in the Patient Register, it increases the chance that exposures were of greater severity. These results may not generalize to injuries that go undocumented by healthcare workers, such as those routinely suffered by amateur and professional sports players (30–32). Finally, by lagging exposure to physical trauma, we decreased the potential for bias due to reverse causation by undiagnosed ALS.

In conclusion, this is, to our knowledge, the first study of its kind to consider age at first physical trauma in relation to ALS risk. We observed an association between traumas earlier in life and increased rate of ALS. This finding may be of particular relevance for prior studies that
have found a higher risk of ALS among both military veterans and some athletes since these populations are at higher risk for traumas, particularly at younger ages (33–37). In addition, in the US over half a million kids between the ages of 0-14 are hospitalized for traumatic brain injuries each year (38). Further studies should consider both the type of physical trauma and age at which physical trauma is experienced, as there may be an etiologic window of heightened susceptibility that has been missed by previous studies.
References


**Web Materials**

*Web Appendix 1*
‘Any trauma’:
Open wounds (ICD-8: 870-884, 890-894, 900-907; ICD-10: S01, S11, S21, S31, S41, S51, S61, S71, S81, S91, T01); fractures (ICD-8: 800-829; ICD-10: S02, S12, S22, S32, S42, S52, S62, S72, S82, S92, T02, T10, T12); dislocations, sprains and strains (ICD-8: 830-848; ICD-10: S03, S13, S16, S23, S33, S43, S46, S53, S56, S63, S66, S73, S76, S83, S86, S93, S96, T03, S290, S390, T064, T095, T112, T115, T132, T135, T143, T146, T733); nerve injuries (ICD-8: 950-959; ICD-10: S04, S14, S24, S34, S44, S54, S64, S74, S84, S94, T04); contusion and crushing, traumatic amputations (ICD-8: 851, 885-887, 895-897, 920-929; ICD-10: S07, S08, S17, S18, S28, S38, S47, S48, S57, S58, S77, S78, S87, S88, S97, S98, T04, T05); and concussion and intracranial injuries (ICD-8: 850, 852-854; ICD-10: S06).

‘Head trauma’:
Concussion and intracranial injuries (ICD-8: 850, 852-854; ICD-10: S06).

‘Other trauma’:
All codes included in ‘Any trauma’ other than concussion and intracranial injuries (ICD-8: 850, 852-854; ICD-10: S06).
Appendix 2

We investigated the association between various trauma exposures and the negative control outcome lung cancer. We are aware of no evidence that physical trauma is causally related to lung cancer. Because smoking is a strong risk factor for lung cancer (1), therefore, an association observed between trauma and lung cancer would suggest that smoking is related to trauma and so raise the possibility of confounding of the trauma-ALS association by smoking. We identified 9,455 cases of lung cancer among the original study’s ALS controls, and sex-, age- and year-matched up to 4 controls to each, for a total of 47,267 individuals (eight lung cancer cases could be matched to only 3 controls). The median age at first recorded diagnosis of lung cancer was 74, and 36% of cases were women.

Table S1 displays the odds ratios for the six exposure-diagnosis combinations and the outcome of lung cancer, in parallel with Table 2.4 in the main text. Only five-year-lagged associations are shown. There was little evidence of an association between trauma and lung cancer for traumas overall, for either inpatient or outpatient visits. There were very few exposed cases of ‘Head’ or ‘Head & Other’ traumas, so these point estimates should be interpreted with caution. That they are generally below 1 suggests that unmeasured confounding due to shared confounding with lung cancer is unlikely.

We also assessed the association between age at first trauma and lung cancer risk, and found significant evidence of bias in a manner similar to the association between age at first trauma and ALS risk. In the youngest group, <35 years of age at first trauma, the estimated odds ratio with lung cancer (OR=1.71; 95% CI 1.20, 2.42) exceeded the odds ratio with ALS (linear trend P=0.003). However, when we split the negative control analysis by type of trauma visit, there was no trend of increasing risk of lung cancer diagnosis with decreasing age at first outpatient trauma (linear trend P =0.398), whereas there was for age at first inpatient trauma (linear trend P =0.019) (Table S2). It should also be noted that if trauma is related to lung cancer because of any factor other than smoking, then the trauma-lung cancer associations we saw in our study may not indicate confounding of the trauma-ALS association by smoking. In addition, the age at head trauma results did not differ by sex. Thus, if smoking is truly only associated with ALS among women, then this might argue that the findings were not confounded by smoking. Future studies of trauma should consider age at the time of the trauma and attempt to account for potential confounding by smoking history.

References

**Web Table 1.** Negative control results. Odds ratios for the association between trauma and lung cancer, 5-year lag.¹

<table>
<thead>
<tr>
<th></th>
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<td></td>
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<td>Cases N (%)</td>
<td>OR* (95% CI)</td>
<td>Controls N (%)</td>
</tr>
<tr>
<td>No Trauma</td>
<td>20877 (96.2)</td>
<td>5103 (96.2)</td>
<td>ref</td>
<td>18484 (85.1)</td>
</tr>
<tr>
<td>Any trauma</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>834 (3.8)</td>
<td>201 (3.8)</td>
<td>0.97 (0.84-1.11)</td>
<td>3227 (14.9)</td>
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<tr>
<td>Head &amp; Other</td>
<td>85 (0.4)</td>
<td>19 (0.4)</td>
<td>0.84 (0.53-1.32)</td>
<td>31 (0.1)</td>
</tr>
<tr>
<td>Other</td>
<td>704 (3.2)</td>
<td>175 (3.3)</td>
<td>1.01 (0.87-1.17)</td>
<td>3166 (14.6)</td>
</tr>
</tbody>
</table>

¹N = 9,455 cases (and 37,812 controls)

*All models adjusted for matching factors (age, sex and calendar date), residence, marital status, and SES.
### Web Table 2. Odds ratios between age at first trauma, by trauma type, and lung cancer (negative control analysis).

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<tbody>
<tr>
<td></td>
<td>Controls N (%)</td>
<td>Cases N (%)</td>
<td>OR* (95% CI)</td>
<td>Controls N (%)</td>
<td>Cases N (%)</td>
<td>OR* (95% CI)</td>
<td>Controls N (%)</td>
</tr>
<tr>
<td>No Trauma</td>
<td>30227 (79.9)</td>
<td>7464 (78.9)</td>
<td>ref</td>
<td>33233 (87.9)</td>
<td>8298 (87.8)</td>
<td>ref</td>
<td>33494 (88.6)</td>
</tr>
<tr>
<td>&lt;35</td>
<td>132 (0.4)</td>
<td>52 (0.6)</td>
<td>1.71 (1.20-2.42)</td>
<td>16 (&lt;0.1)</td>
<td>2 (&lt;0.1)</td>
<td>0.42 (0.09-2.08)</td>
<td>119 (0.3)</td>
</tr>
<tr>
<td>35-54</td>
<td>1977 (5.2)</td>
<td>561 (5.9)</td>
<td>1.13 (1.02-1.26)</td>
<td>726 (1.9)</td>
<td>200 (2.1)</td>
<td>1.03 (0.86-1.24)</td>
<td>1450 (3.8)</td>
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<td>55-74</td>
<td>4713 (12.5)</td>
<td>1184 (12.5)</td>
<td>0.99 (0.93-1.07)</td>
<td>3196 (8.5)</td>
<td>807 (8.5)</td>
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<td>2405 (6.4)</td>
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<td>75+</td>
<td>763 (2.0)</td>
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<td>1.01 (0.85-1.19)</td>
<td>641 (1.7)</td>
<td>148 (1.6)</td>
<td>0.85 (0.69-1.03)</td>
<td>344 (0.9)</td>
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</tbody>
</table>

*All models adjusted for matching factors (age, sex and calendar date), residence, marital status, and SES.
CHAPTER III

ALS AND THE MILITARY: A POPULATION-BASED STUDY IN THE DANISH REGISTRIES

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ABSTRACT

**Background:** Prior studies have suggested that military service may be associated with the development of amyotrophic lateral sclerosis. We conducted a population-based case-control study in Denmark to assess whether occupation in the Danish military is associated with an increased risk of developing amyotrophic lateral sclerosis.

**Methods:** There were 3,650 incident cases of amyotrophic lateral sclerosis recorded in the Danish National Patient Registry between 1982 and 2009. Each case was matched to 100 age- and sex-matched population controls alive and free of amyotrophic lateral sclerosis on the date of the case diagnosis. Comprehensive occupational history was obtained from the Danish Pension Fund database, which began in 1964.

**Results:** 2.4% (n=8,922) of controls had a history of employment in the military prior to the index date. Military employees overall had an elevated rate of ALS (OR=1.3; 95% CI: 1.1-1.6). A ten-year increase in years employed by the military was associated with an odds ratio of 1.2 (95% CI: 1.0-1.4%), and all quartiles of time employed were elevated. There was little suggestion of a pattern across calendar year of first employment, but there was some evidence that increasing age at first employment was associated with increased ALS rates. Rates were highest in the decade immediately following the end of employment (OR=1.6; 95% CI: 1.2-2.2).

**Conclusions:** In this large population-based case-control study, we find that employment by the military is associated with increased rates of ALS. These findings are consistent with earlier findings that military service or employment may entail exposure to risk factors for ALS.
MAIN TEXT

INTRODUCTION

The causes of amyotrophic lateral sclerosis (ALS) are largely unknown. Nearly a decade after the first Gulf War in 1990-1991, studies began to document an association between military service and risk of ALS.\textsuperscript{1-4} Two studies of Gulf War veterans observed a near-doubling of ALS rates in young veterans who had been deployed in the Gulf\textsuperscript{2,3}, while the large Cancer Prevention Study II (CPS-II) found a 50\% increase in self-reported veterans.\textsuperscript{4} An analysis of the National Longitudinal Mortality Study (NLMS), a large representative sample of the non-institutionalized U.S. population, found a hazard ratio of 1.21 (95\% CI: 0.97-1.50) for ALS among all veterans, and a ratio of 1.46 (95\% CI: 1.13-1.88) among WWII veterans.\textsuperscript{5} In the only non-U.S. study to date, the rate of ALS in French military service members (rate = 1.7/100,000 per year), was lower than that of the general population, but the study was limited by its small size (n=73 ALS patients) and by its case ascertainment, and elevated rates were observed in those aged 40-54.\textsuperscript{6} A recent review of thirty articles and abstracts concluded that “although current literature suggests a positive association between military service and ALS/MND etiology, it is too limited to make definitive statements.”\textsuperscript{7}

Several factors may explain a causal relationship between military service and an increased risk of ALS. Exposures that are more common in military service-members and that have been tentatively associated with ALS include: insecticides\textsuperscript{8,9}, aerosolized lead\textsuperscript{8,10}, traumatic injury\textsuperscript{11-13}, viral infections\textsuperscript{14,15}, and intense physical activity\textsuperscript{16,17}.

In the present study we used Danish registry data covering 27 years of ALS diagnoses to perform the largest analysis to date on the link between military occupations and ALS risk; this is also the first study outside the U.S. performed in a population representative sample.
METHODS

Case Ascertainment

We obtained cases from the Danish National Patient Registry, using primary discharge diagnoses of 348.0 (ICD-8) or G12.2 (ICD-10). ICD-8 codes were used in Denmark through 1993, with ICD-10 thereafter. All hospital admissions nationwide are captured by the National Patient Registry, beginning on January 1, 1977. In a validation sub-study of 173 ALS cases identified this way, we obtained medical records and confirmed the ALS diagnosis in 160 (92.5%). We found no significant difference in predictive validity between ICD-8 and ICD-10 codes in our validation sub-study. An earlier validation sub-study in Denmark found confirmed ALS in only 68% of hospital discharge-identified records, but was based on only 25 cases and was limited to ICD-10 ALS codes.

We limited our case definition to first diagnoses on or after January 1, 1982, a five-year washout period to reduce the inclusion of prevalent cases. Case ascertainment was performed through December 31, 2009, and the index date was the first recorded hospitalization with ALS recorded as the primary discharge diagnosis.

Control Selection

We obtained controls from the Central Person Registry, which covers all residents in Denmark since 1968. We selected 100 controls for each case, individually matched on sex, age in 1-year windows, and who were free of an ALS diagnosis in the Patient Registry as of the index date (risk-set sampling). All inhabitants of Denmark are assigned a unique 10-digit Central Person number, which includes information on date of birth and sex, and can be used to link between multiple databases, including the Central Person Registry, Danish National Patient Registry, and the Danish Pension Fund (see below).
Covariates

In addition to the matching variables of age, sex, and calendar date, we obtained information on job title, spouse’s job title, marital status, and area of residence on the index date from the Central Person Registry. Job title was classified into five groups as a proxy for socioeconomic status (SES): academics and managers (1), high salaried (2), low salaried (3), skilled workers (4) and unskilled workers (5). When both an individual and his or her spouse’s job title category were reported, we used the higher SES category (lowest number) of the two. If neither was reported the individual’s job title was considered missing, categorized using a missing indicator. Marriage status was categorized as married, unmarried, divorced, or widowed as of the index date. Area of residence was classified as Copenhagen/Aarhus/Odense (the three largest cities in Denmark), Other Denmark/Greenland, or Unknown.

Occupational History

We obtained employment histories from the Danish Pension Fund databases, which maintains employment data on all wage earners aged 16-66, beginning in 1964. In the pension database each employment is recorded with start and end dates, the Central Person number of the employee, and a unique 8-digit company number determined by the tax authorities. The company number is based on its main activities as classified by Statistics Denmark, which employs an extended version of the International Standard Industrial Classification codes. For the present analysis we focused on: Air force, Marine, Army, Civil Defense, Home Guard, and the Royal Army, and Defense not elsewhere classified. The latter do not differentiate between Air force, Marine and Army. We calculated the total number of years employed by each branch. A record of employment by the Danish military in the pension database reflects only that the employee was paid by the military, a definition that may include both military and civilian
employees. Military service in Denmark is compulsory for all men who meet a minimal level of
overall health, and has been limited to less than one year since 1973 with some rare exceptions,
e.g. the royal guard. Because pension records – and thus our ability to classify someone as
employed by the military – begin only after one year of employment in the military, our
exposure definition excludes those who only served their compulsory amount.

Our primary analysis consisted of whether or not an individual had ever worked in the
military (or a particular branch) prior to the index date. We analyzed amount of time spent in
each occupation by quartiles of time worked in each branch, and as a continuous number of
years. For each job, we also determined the decade and the age during which the individual was
first employed. Finally, we categorized the number of years between the index date and the last
year of employment in the military.

**Statistical Analysis**

All models were fit via conditional logistic regression, with strata defined by the age-, sex-, and calendar year-matched sets. We categorized the year first employed in a particular
employment history began in 1964, any individual employed prior to 1964 and still employed at
that time would be included in the ≤1964 category; the category is thus a combination of
prevalent and incident “hires”. We categorized the number of years employed in the military
based on the distribution of number of years worked among cases: ≤1964, 1.0-2.4, 2.4-4.7, 4.7-
12.4, ≥12.4. Results were unchanged when the quartiles were based on the distribution among
controls. We were forced to use a category for ≤1964 because the age at which an individual was
first employed prior to 1964 is unknown, only that they were employed at that time and
potentially some amount of time earlier. We can therefore not accurately calculate the total number of years worked (or age first worked) for those individuals. We categorized the age at which an individual was first employed by the military as: ≤1964, <21, 21-29, and 30+. The category for ≤1964 was used for the reasons described above. We categorized the number of years between the year of last employment in the military and the index date as: 0-9, 10-19, 20-29, and 30+, with no military occupation serving as the reference category. We modeled number of years employed by the military and age at first military employment linear to assess trends. For years employed we used an indicator variable to identify those whose employment began before 1964 and set their time of employment as a fixed value, and for age at first military employment we used an indicator to identify those who never worked for the military, and set their age of first employment as a fixed value. This has the effect of only modeling the relationship among those with known or well-defined values, but without destroying the nature of the matched case-control study.

In addition to the matching factors, all models were adjusted for SES. Inclusion of area of residence as of the index date did not change the estimates for any of the exposures of interest, so we omitted residence from the final models. As a sensitivity analysis we further adjusted our analyses for time spent in the military; point estimates were largely unchanged. We assessed departures from linearity for continuous measures via penalized cubic splines in R, with the penalty chosen by Akaike Information Criteria.23

Finally, we employed a quantitative sensitivity analysis to assess the potential for a dichotomous confounder, such as smoking behavior, to explain our results.24 We based the characteristics of the putative confounder on a previously published meta-analysis of the
association between smoking and ALS,\textsuperscript{25} and based confounder prevalence rates on available data on smoking in Denmark.\textsuperscript{26}

**RESULTS**

Between January 1, 1982 and December 31, 2009 there were 3,650 diagnosed cases of ALS in Denmark. Table 3.1 shows descriptive statistics for controls with and without a history of military occupations. Military employees were more likely to be male and were younger than non-military. Military employees were also more likely to reside in the three large cities in Denmark, have higher SES, and be married than non-military.

Among controls, 2.4% (n=8,922) had a history of employment in the military prior to the index date. Military employees overall had an elevated rate of ALS diagnosis (OR=1.3; 95% CI: 1.1-1.6) (Table 3.2). There was little evidence of a difference by branch, though small numbers in the marines and army limit comparisons. Because there was a total of only one case with a history of Civil Defense, Home Guard, or Royal Guard occupation, we did not report results for these branches individually. There was little evidence of effect modification of military service (any branch) by sex; all sex-stratified measures were similar with broadly overlapping confidence intervals.
Table 3.1. Descriptive statistics of controls, Denmark January 1 1982 – December 31 2009, separated by ever having a history of military occupation. Characteristics describe the controls on the case (index) date.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Military (n=8,922)</th>
<th>Non-Military (n=356,078)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>7507 (84)</td>
<td>187893 (53)</td>
</tr>
<tr>
<td>Year of birth, µ (sd)</td>
<td>1940 (14)</td>
<td>1932 (14)</td>
</tr>
<tr>
<td>Age (years), n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>970 (11)</td>
<td>19030 (5.3)</td>
</tr>
<tr>
<td>45-54</td>
<td>1723 (19)</td>
<td>40277 (11)</td>
</tr>
<tr>
<td>55-64</td>
<td>2860 (32)</td>
<td>89740 (25)</td>
</tr>
<tr>
<td>65-74</td>
<td>2351 (26)</td>
<td>122749 (35)</td>
</tr>
<tr>
<td>74-85</td>
<td>934 (11)</td>
<td>74766 (21)</td>
</tr>
<tr>
<td>≥85</td>
<td>84 (0.94)</td>
<td>9516 (2.7)</td>
</tr>
<tr>
<td>Residence, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen/Aarhus/Odense</td>
<td>4291 (48)</td>
<td>157758 (44)</td>
</tr>
<tr>
<td>Other Denmark/Greenland</td>
<td>4623 (52)</td>
<td>197130 (55)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8 (0.09)</td>
<td>1190 (0.33)</td>
</tr>
<tr>
<td>SES*, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 – High</td>
<td>2141 (24)</td>
<td>33332 (9.4)</td>
</tr>
<tr>
<td>2</td>
<td>974 (11)</td>
<td>39353 (11)</td>
</tr>
<tr>
<td>3</td>
<td>1477 (17)</td>
<td>64518 (18)</td>
</tr>
<tr>
<td>4</td>
<td>2278 (26)</td>
<td>97102 (27)</td>
</tr>
<tr>
<td>5 – Low</td>
<td>1391 (16)</td>
<td>71584 (20)</td>
</tr>
<tr>
<td>Unknown</td>
<td>661 (7.4)</td>
<td>50189 (14)</td>
</tr>
<tr>
<td>Marriage Status, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>6205 (70)</td>
<td>219082 (62)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>827 (9.3)</td>
<td>32645 (9.2)</td>
</tr>
<tr>
<td>Divorced</td>
<td>1121 (13)</td>
<td>36755 (10)</td>
</tr>
<tr>
<td>Widower</td>
<td>769 (8.6)</td>
<td>67596 (19)</td>
</tr>
</tbody>
</table>

*SES: socioeconomic status
Table 3.2. Odds ratios for history of employment in the military (overall) and by branch.

<table>
<thead>
<tr>
<th>Branch</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>OR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Military</td>
<td>3534 (97)</td>
<td>356078 (98)</td>
<td>ref</td>
</tr>
<tr>
<td>Any Military</td>
<td></td>
<td></td>
<td>1.3 (1.1-1.6)</td>
</tr>
<tr>
<td>Air Force</td>
<td>116 (3.2)</td>
<td>8922 (2.4)</td>
<td>1.5 (1.0-2.3)</td>
</tr>
<tr>
<td>Marines</td>
<td>9 (0.25)</td>
<td>570 (0.16)</td>
<td>1.6 (0.84-3.2)</td>
</tr>
<tr>
<td>Army</td>
<td>24 (0.66)</td>
<td>1537 (0.42)</td>
<td></td>
</tr>
<tr>
<td>Defense NEC</td>
<td></td>
<td></td>
<td>0.70 (0.37-1.3)</td>
</tr>
<tr>
<td></td>
<td>98 (2.7)</td>
<td>7261 (2.0)</td>
<td>1.3 (1.1-1.7)</td>
</tr>
</tbody>
</table>

*Adjusted for matching factors and SES
**NEC = Not Elsewhere Classified
Table 3.3 displays the results for total number of years employed in the military. Quartiles were defined from cases with a history of post-1964 employment in the military. ALS rates were elevated for all quartiles, though sparse numbers resulted in wide confidence intervals. When the quartiles were further collapsed, the odds of ALS in those who worked more than 2.1 years (the first quartile cut-point) was 1.5 times the odds in those who worked less than 2.1 years (95% CI 1.1-1.9). When modeled linearly, each ten-year increment in number of years worked (which roughly corresponds to the IQR in ever-employed cases; see Table 3.3) for the military was associated with an odds ratio of 1.2 (95% CI 1.0-1.4%). Visual inspection of flexible splines indicated no substantial departures from linearity or threshold effects.

The association did not vary much by year first worked in the military (Table 3.3), although decreasing numbers limited our ability to discern differences in more recent decades. We observed some evidence that military employment begun after the age of 21 was more highly associated with ALS than earlier service (Table 3.3). When modeled linearly however, each five-year increment in age-at-first-military-employment was associated with a null odds ratio of 1.0 (95% CI 0.97-1.1). Further adjustment for number of years in the military, entered in quartiles, did not substantially change the point estimates.

When we categorized individuals by how long it had been since their last military employment, there was a suggestion that employment in the decade prior to the index was more strongly associated with ALS (Table 3.3). Military employment in the decade prior to the index date was associated an odds of ALS 1.6 times higher than those who did not work for the military (95% CI 1.2-2.2), whereas military employment more than a decade prior to the index date was associated with only a an odds ratio of only 1.1 ALS (95% CI 0.90-1.5).
Table 3.3. Odds ratio by number of years in a military occupation.

<table>
<thead>
<tr>
<th>Number of years employed by military</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
<th>OR**</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3534 (97)</td>
<td>356078 (98)</td>
<td>ref</td>
</tr>
<tr>
<td>Pre-1964***</td>
<td>49 (1.3)</td>
<td>3873 (1.1)</td>
<td>1.2  (0.93-1.6)</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; Quartile</td>
<td>16 (0.44)</td>
<td>1512 (0.41)</td>
<td>1.1  (0.65-1.8)</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; Quartile</td>
<td>17 (0.47)</td>
<td>1126 (0.31)</td>
<td>1.5  (0.95-2.5)</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; Quartile</td>
<td>17 (0.47)</td>
<td>1212 (0.33)</td>
<td>1.4  (0.89-2.3)</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Quartile</td>
<td>17 (0.47)</td>
<td>1199 (0.33)</td>
<td>1.4  (0.87-2.3)</td>
</tr>
<tr>
<td>Calendar year of first employment by military</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3534 (97)</td>
<td>356078 (98)</td>
<td>ref</td>
</tr>
<tr>
<td>≤ 1964</td>
<td>49 (1.3)</td>
<td>3873 (1.1)</td>
<td>1.2  (0.93-1.7)</td>
</tr>
<tr>
<td>1965-1974</td>
<td>39 (1.1)</td>
<td>2797 (0.77)</td>
<td>1.4  (1.0-1.9)</td>
</tr>
<tr>
<td>1975-1984</td>
<td>18 (0.49)</td>
<td>1500 (0.41)</td>
<td>1.2  (0.76-2.0)</td>
</tr>
<tr>
<td>1985-1994</td>
<td>9 (0.25)</td>
<td>564 (0.15)</td>
<td>1.6  (0.83-3.2)</td>
</tr>
<tr>
<td>1995-2009</td>
<td>1 (0.03)</td>
<td>188 (0.05)</td>
<td>0.53 (0.07-3.8)</td>
</tr>
<tr>
<td>Age (years) at first employment by military</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3534 (97)</td>
<td>356078 (98)</td>
<td>ref</td>
</tr>
<tr>
<td>Pre-1964***</td>
<td>46 (1.3)</td>
<td>3415 (0.94)</td>
<td>1.3  (1.0-1.8)</td>
</tr>
<tr>
<td>&lt;21</td>
<td>14 (0.38)</td>
<td>1812 (0.50)</td>
<td>0.78 (0.45-1.3)</td>
</tr>
<tr>
<td>21-29</td>
<td>33 (0.90)</td>
<td>2055 (0.56)</td>
<td>1.6  (1.1-2.3)</td>
</tr>
<tr>
<td>30+</td>
<td>23 (0.63)</td>
<td>1640 (0.45)</td>
<td>1.4  (0.94-2.1)</td>
</tr>
<tr>
<td>Number of years since final employment by military</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3534 (97)</td>
<td>356078 (98)</td>
<td>ref</td>
</tr>
<tr>
<td>0-9</td>
<td>46 (1.3)</td>
<td>2800 (0.77)</td>
<td>1.6  (1.2-2.2)</td>
</tr>
<tr>
<td>10-19</td>
<td>20 (0.55)</td>
<td>2186 (0.60)</td>
<td>0.91 (0.58-1.4)</td>
</tr>
<tr>
<td>20-29</td>
<td>27 (0.74)</td>
<td>2067 (0.57)</td>
<td>1.3  (0.89-1.9)</td>
</tr>
<tr>
<td>30+</td>
<td>23 (0.63)</td>
<td>1869 (0.51)</td>
<td>1.2  (0.82-1.9)</td>
</tr>
</tbody>
</table>

*Quartile cut points calculated from cases with non-zero employment: 2.1, 4.7, 12.4 years

**Adjusted for matching factors (age, sex, index date) and SES

***The number of years of employment and the age at first employment can only be definitively established for those who began work after 1964
Sensitivity Analysis

We employed a sensitivity analysis to assess the potential for a single dichotomous exposure (smoking) to explain our results. A meta-analysis for the association between smoking and ALS reported a pooled rate ratio of 1.3. With an estimated 30% of the population as ever-smokers in 2000, we varied the prevalence of smoking in those employed by the military to assess the stability of our results. Our best estimate of the smoking prevalence in the Danish military is 34% as reported in the survey of military personnel. This resulted in a biased OR of only 1.01. If the smoking prevalence in military employees was twice as high (60%) the OR would be 1.08. In order to get a biased OR as large as what we found in this paper (1.3), the odds ratio linking ALS and smoking would have to be as high as 1.6, which is considerably higher than the pooled estimate, particularly that for males, and smoking prevalence among Danish military employees would have to be 90%.
DISCUSSION

In our study, employment in the Danish military was associated with a 1.3-fold increased rate of ALS, with some evidence of a trend with increasing years of employment. Additionally, we found some evidence that Air Force and Marine employees were at a heightened risk for ALS, although given the numbers in the different categories, it is not clear whether a true difference by branch exists. There was no strong evidence for increased risk in any particular period of employment or age at first employment. Our current findings are the first outside the U.S. to use a population-representative sample and we find results similar to prior U.S.-based studies.

Five prior studies have assessed ALS risk in relation to military service. The first, in Gulf War veterans, reported a standardized mortality ratio of 2.27 (95% CI 1.27-3.88) for ALS diagnosed before the age of 45 in a three-year period from 1995 to 1998. This study was based in a cohort of 695,000 United States service members who served in the region of the Gulf War between August 1990 and April 1991, with case ascertainment through a variety of methods, including informal ties, Department of Defense, Department of Veterans Affairs, and the ALS association registries. A second study compared deployed to non-deployed Gulf War era veterans, and similarly found roughly a doubling in risk of ALS (RR=1.92; 95% CI 1.29-2.84). A third study in the national CPS-II cohort of roughly 500,000 men found that a self-reported history of service (“Were you in the US Armed Services?”) in the US Armed Forces was associated with a risk ratio of 1.53 (95% CI 1.12-2.09). A large study in a U.S.-representative sample of self-reported military service found a similar association to the current study. Finally, a study of 73 French military service members with ALS found a slightly decreased rate of ALS in former service members overall as compared to the general population, although the study was
limited by the fact that cases were limited to those still receiving military health care, creating the potential for downward selection bias. Despite this, elevated rates were observed in those aged 40-54.6

There is limited data on what is involved with employment in the military in Denmark. Currently, a survey done by the Danish Cancer Society in 2004 on the risks associated with military radar equipment surveyed 1,125 men without cancer employed by the Danish military.27 While the survey was conducted in a significantly younger cohort of military employees, the results indicated that roughly one third reported exposure within or outside the military to welding, metal processing, or organic solvents. Twenty-eight percent reported daily smoking, with a further 34% reporting a history of smoking. This is nearly the same as the overall reported smoking prevalence in Denmark of 30% in 2000 in those over the age of 15.26 The prior U.S. studies of Gulf War veterans looked specifically at deployed veterans with the suggestion that some aspect of deployment accounts for the increased risk of ALS.2,3,14,28 Only about one thousand Danish military service members per year have been deployed in recent decades, and the circumstances of those who are deployed are somewhat different than for U.S. military personnel. For example, deployed Danish military personnel rarely see combat prior to the war in former Yugoslavia in the 1990’s. Thus, it seems unlikely that some aspect of deployment would account for our findings among Danish military workers.

A further analysis of the Gulf War veterans study found that the increased risk of ALS in deployed veterans had returned to that of non-deployed veterans with ten years after deployment.28 We observed a similar pattern, although weaker. The first decade after employment was associated with the highest relative increase in ALS, although the rate of ALS remained elevated after a decade.
Occupation in the military may entail exposure to several factors that could explain a true association between military work and an increased risk of ALS. While few exposures have been definitely linked to ALS, some exposures that have been suggested to be linked to ALS and are more common in the military include: insecticides\textsuperscript{8,9}, aerosolized lead\textsuperscript{8,10}, traumatic injury\textsuperscript{11–13}, viral infections\textsuperscript{14,15}, and intense physical activity.\textsuperscript{16,17} Military workers are also potentially exposed to organic solvents and formaldehyde, both of which have been suggested as linked to ALS.\textsuperscript{27,29,30}

The present study is limited by the lack of military occupation history prior to 1964. In general, however, we would expect bias from this type of exposure misclassification to be towards the null, which could further explain the slightly lower effect estimates we observed in comparison with the U.S. studies. We were also unable to adjust for smoking, which is a suspected risk factor for ALS\textsuperscript{31,32,25}, although the evidence is weaker among men, who make up most of the Danish military employees\textsuperscript{25,33}. However, Weisskopf et al. did not observe a change in their estimates when adjusting for smoking in the CPS-II study. Furthermore, unlike in the U.S., in Denmark smoking rates of military workers are comparable to those in the general population thus greatly reducing any possible confounding.\textsuperscript{27} When we employed a quantitative sensitivity analysis, there was little evidence that realistic confounding by smoking could explain our observed results. Employment by the military in Denmark involves many activities in addition to traditional military services, but indirect data suggests that several suspected risk factors for ALS may be fairly common among the Danish military employees.\textsuperscript{27}

A strength of this study is the structure of the healthcare system in Denmark. Prior findings in the U.S. may have been influenced by the fact that veterans in the U.S. were historically more likely to have access to healthcare, potentially leading to differential ALS
ascertainment. Denmark has had universal healthcare coverage since the 1930s, greatly limiting the possibility that access to care is driving our findings.

Associations between military work and ALS have now been reproduced in multiple large studies of differing design. Future studies should focus on collecting more detailed information, particularly on potential confounders of smoking and physical activity, and on potential specific risk factors including what kind of military work is undertaken and what types of exposures are accrued. Such studies might be accomplished via prospective case-control studies with data collection from military databases.

In summary, we found evidence for a link between Danish military service and ALS risk in a large, population-based study. It is, to our knowledge, the first registry-based study using objective records of military employment and ALS diagnosis, and the first population-representative study outside of the U.S. Our findings underline the importance and value in working within military populations to identify specific risk factors that could account for the increased risk of ALS among military personnel.
REFERENCES


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